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STUDIES ON THE BIOLOGY OF THE PARASITES OF THE PEA LEAF MINER *PHYTOMYZA ATRICORNIS* (MEIGAN)

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Introduction

The leaf miner *Phytomyza atricornis* (Meigan) is a serious pest of several crops of economic importance. The host range is considerable and Ahmad and Gupta (1940) have listed twenty-nine species of plants, comprising fourteen families. The leaf miner is cosmopolitan in distribution and is widespread throughout Europe including Russia, United States of America and other countries of the old and new world. Lefroy (1906) was the first to record the pest on cruciferous plants in India. A review of published literature shows that the biology of no parasite of any leaf miner has been studied in India so far. This is not surprising because the problem is very difficult and beset with many difficulties. Even outside India there are only very few contributions on the biology of the parasites of leaf miners. We may now briefly review this work.

The investigations of Voukassovitch (1928) and Cohen (1936) on the parasites of the leaf miner *Phytomyza atricornis* are outstanding. Ahmad and Gupta (1941) while studying the biology of *Phytomyza atricornis* recorded *Solenotus* sp. as an endoparasite of the pest which is not actually the fact. In this paper the bionomics and biology of *Solenotus* sp. new an ectoparasite and *Rhopalotus* sp. new* (Eulophidæ: Hymenoptera) an endoparasite are described in detail. The fascinating problem on the parasite complex involving multiparasitism between the chalcid *Rhopalotus* and the braconid (to be identified) and its effect on the development of the latter is also discussed.

Material and Methods

The biology of the parasite was studied under room temperature and humidity. The mean maximum and minimum temperature being 75·5 and 79° F. during the period from fifteenth February to twenty-fourth March when investigations were carried out on the biology of the parasite.[5] Leaves

* The description of the new species is being published elsewhere.

infested with leaf miners were collected and reared for parasite emergence. The parasites were offered with fresh field collected hosts in small tubes and after parsitisation the leaves containing the parasitised hosts were removed to petri dishes which were kept moist by keeping wet filter-paper at their base. The tracheal system was studied by temporary mounting in glycerine.

BIONOMICS OF *Solenotus* SP. NEW

The adults of *Solenotus* sp. make their appearance in the field from the second week of February to the middle week of March, attacking the leaf miners on pea and lucerne. From April onwards the parasite population decreases. It is probable that the peak of adult emergence occurs between the first and second week of March.

Host Selection

The female parasite actually flies about the leaf until she locates the suitable stage of the host larva in the tunnel of the leaf. Generally the parasite selects only well-developed larva for parasitisation. As soon as the parasite locates the host she pushes her ovipositor through the membranous covering of the leaf that protects the larva, stings and paralyses the host and deposits generally only one egg on the body. Occasionally up to three eggs are laid. But this is rather rare. The parasite larva after hatching is so tiny that it cannot be seen on the body of the host except under the low power objective of a microscope. In all the dissections carried out the host larvæ were invariably dead even though the parasite larva had apparently just hatched. Even under superparasitised conditions the parasite larvæ of which only a maximum of three were observed during the course of these investigations, completed their development and emerged as adults. Pupation takes place within the mine of the host but always away from the remains of the host body.

Development

During the growth and development of the immature stages of *Solenotus* sp., in all there are five instars. The main points of difference between the various instars are size, shape of mandibles, the tracheal system and the number of spiracles.

(*a*) *Egg* (Plate VIII, Fig. 5).—The egg is transparent and oval elongate in shape. The egg measures 0·35 mm. in length. The chorion is very smooth without any reticulations. The egg is deposited externally on the body of the host. The percentage viability of the egg is very high. The incubation period varies from fifteen to twenty-four hours.

(*b*) *First instar* (Plate VIII, Fig. 6).—The freshly hatched larva is composed of a head and body segments of which twelve are clearly differentiated. The thirteenth and fourteenth segments are not at all clearly defined. The larva is translucent in colour with a tendency to light green in older individuals. The shape is subcylindrical with maximum breadth about the middle, tapering anteriorly into a bluntly rounded structure and posteriorly into a bifurcated papillæ. Spines are present on all the body segments except the first two and the last segments. All segments are devoid of spicules.

The respiratory system makes its appearance at this stage itself. There are two lateral longitudinal trunks with spiracleas only in the segments fourth, sixth, seventh, eighth and the ninth. So in all there are five pairs of spiracles in the first instar unlike most other chalcids which usually have only four pairs. The mandibles are sharp and slightly curved. They are pale brown in colour and overlap each other at their tips. The first instar larva measures about 0·35 mm. in length.

(*c*) *Second instar*.—The second instar larva is similar to the first instar except that it is slightly larger in size and without the caudal papillæ. As the thoracic segments have increased in length and width the head appears proportionately smaller. The individual body segments are more conspicuous and translucent and greenish white. The respiratory system is well developed. The two lateral longitudinal trunks are quite thick and prominent. The full component of eight pairs of spiracles are present. The spines which were present in the first instar are absent in this instar. The mandibles are larger than in the first instar. The second instar larva measures 0·425 mm. in length.

(*d*) *Third instar*.—The third instar larva very much resembles the second except that it is again slightly larger than the second. It is greyish white in colour except at the region of the alimentary canal which is pale brown.

The respiratory system is more developed. Numerous lateral branches are given off to the internal organs and to the nerve cord. Mandibles are heavily chitinized, with the apex more closely set together than in the previous instars. Specimens referable to this instar have body length varying from 0·70 to 0·75 mm.

(*e*) *Fourth instar*.—The fourth instar closely resembles the previous stadium but can be distinguished from the third instar by the mandibular measurements. The alimentary canal is larger and its contents tend to give the larva a pale colour. The fat bodies appear at this stage.

The respiratory system is well developed with anastomosing branches. Setæ are absent. Mandibles are quite strong. Specimens referable to this instar measure from 1·05 to 1·075 mm. in body length.

(*f*) *Fifth instar* (Plate VIII, Fig. 7).—Final instar larvæ are light green in colour and the alimentary canal is pale brown. The head is a rigid hemispherical capsule, on which the antennæ are represented by a pair of conspicuous sensillæ. A few lesser developed sensillæ are also seen.

Numerous rows of spicules make their appearance in this instar. Except the head segment, spicules are arranged in regular rows in each segment. The ventrum is devoid of spicules. Mandibles are heavily chitinized and strong. The tracheal and spiracular trunks get more wide and prominent with numerous branches anastomosing and ramifying throughout the body.

The larva when full fed moves away from the host remains and pupate in the gallery made by the host. Specimens referable to this instar have a body length ranging from 1·25 to 1·30 mm. During the growth and development the larvæ exclusively feed on the blood of the host and do not attack the internal organs.

(*g*) *Pupa* (Plate VIII, Fig. 8).—Pupa is of the naked chalcid type. Initiation of the pupal phase is marked by the passing of fœcal pellets for the first time during the development. The body of the larva becomes cylindrical and rapid changes begin. The developmental changes can be observed closely under the low power objective of a microscope. This can even be seen to some extent with the naked eye. A slight constriction appears in between the head and the thorax and the thorax and the abdomen. The eyes and other appendages appear gradually. The early pupa is light green in colour with red eyes. As it grows older the colour of the body gets changed to a metallic lustre and the eyes become deep red and prominent. The thoracic region develops conspicuous imaginal wing buds. The average duration of the pupal phase is about five days at laboratory temperature and humidity. Specimens of pupa measure from 1·45 to 1·55 mm. in length.

(*h*) *The adult* (Plate VIII, Fig. 9).—The adult cuts a hole through the membranous gallery constructed by the host and emerges out. It rests for some time and flies away. The colour of the body is metallic green with red iridescence.

Length of the life-cycle.—The period from the egg stage to the adult of the parasite varies from ten days to fourteen at room temperature and humidity in the month of March. The incubation period of the egg varies from fifteen to twenty-four hours. The period from hatching of the egg to

pupation lasts from five to seven days and from pupation to emergence of the imago four to six days.

Longevity.—In an experiment with six females which were given freshly cut raisins as food, the length of life varied from fifteen to eighteen days under room temperature and humidity in the month of March.

Sex ratio.—Leaves infested with leaf miners were collected and the emergence of parasites were closely observed. Under field conditions the females predominated, their percentage being 90, males constituting just 10 per cent. of the total emergence.

Percentage of Parasitism

The percentage of parasitism is very high. 177 infested leaves were picked from the fields at random and the number of host miners counted on them. It was found that there were 396 hosts on these leaves. The parasites on them were allowed to emerge under room temperature and humidity, and the adults were counted every day. It was observed that the number of parasites that emerged were fairly high and counted 310. So the percentage of parasitism is very high. It was also observed that two other parasites, *viz.*, *Rhopalotus* sp. and a braconid were also parasitising the host. Out of the total emergence *Solenotus* sp. constituted 90 per cent., *Rhopalotus* 9 per cent. and the Braconid 1 per cent.

Morphology of Immature Stages

Chætotaxy (Plate IX, Fig. 1).—The spines are seen in the first instar and the spicules in the fifth. Spines are present on all the body segments except the first two and the last. Two longitudinal rows can be seen running from the third segment to the thirteenth, one on each side of the body. There are two transverse bands of very minute spines on the segment fifth and sixth. Each band consists of six spines. In addition to this there can be seen one pair on each side of the segments seventh, tenth and thirteenth. So in all there are eighteen pairs of spines present on the body of the first instar larva.

Numerous minute spicules make their appearance in the final instar. Except the head segment, these spicules are arranged in regular rows.

Mouth parts (Plate IX, Fig. 2).—Mandibles are extremely sharp and slightly curved. The tip is pale brown and the rest of the mandible being of a very pale yellow colour. They overlap at their tips. Labrum is provided with sensillæ. The epistoma is well developed and is a dome-shaped structure covering the mouth parts. The mandibles articulate with the supra and infra pleurostomal rami on either side. In the upper articulation the condyle

is on the ramus while in the lower the order is reversed, the condyle being a hollow pit on the pleurostoma. The tentorial rods give strength to the head capsule. Facial rods which are well developed in the case of other ectophagous parasites are not so well developed in this case and are much simplified. Morris (1935) has described similar mouth parts and facial rods in *Microplectron fuscipennis* Zett.

Respiratory system (Plate IX, Figs. 3 and 4).—The respiratory system consists of two lateral longitudinal trunks, united anteriorly but not posteriorly. There are eight pairs of spiracles unlike most other chalcids which have nine pairs. The spiracles are present in the segments third, fourth, sixth, seventh, eighth, ninth, tenth and eleventh. So they are regularly arranged from segment third to eleventh with the exception of fifth which forms a gap. They are attached to the lateral longitudinal trunks by means of coiled spiracular tubes.

In the second segment where the two trunks meet, five branches are given off on either side. In each spiracular segment with the exception of fifth, the traceales give off a pair of branches, one on either side of it, which in their turn bifurcate and further branch. Segment fifth, which is a non-spiracular segment, also receives these branches from the main trunk.

Digestive System (Plate IX, Fig. 5).—Mouth leads into a short, narrow pharynx, which in its turn leads into the stomach. The latter is quite large and occupies most of the body cavity. The mid-intestine becomes a distinct hind-intestine at the twelfth segment and the hind gut opens out as anus.

Salivary glands are well developed. But the silk glands as in the case of other ectophagous parasites, which spin cocoon, are not well developed.

The nervous system (Plate IX, Fig. 5).—The nervous system consists of a well-developed brain and a ventral nerve cord. There is a sub-œsophageal ganglion, three thoracic and six abdominal. The ventral nerve cord does not extend beyond the tenth segment. The last abdominal ganglion is a composite structure that represents the ganglia of the remaining four abdominal segments.

BIOLOGY OF *Rhopalotus* SP. NEW

Seasonal History

The emergence of adult *Rhopalotus* sp. commences in the second week of March and continues up to the last week. It is, however, probable that the peak of adult emergence occurs about March third week to last week, as during this period the population of the parasite is high in the field. *Solenotus* sp. makes its appearance first and *Rhopalotus* afterwards.

Host Selection

The female parasites move about over the leaf until they locate the suitable stage of host larva in its mine. Usually the parasite selects chrysalid stages of the pest but at times it attacks the last instar larva also. In the former case the development of the parasite is completed while in the latter case the developing grub of the parasite allows the host to form puparia whereas the parasite itself hibernates till the next spring. As soon as the parasite locates the host she pierces its ovipositor first through the membranous gallery of the host and then through the body of the host, and deposits one to six eggs at a time into the body. However, only one individual completes its development and emerges as adult. The supernumerary larvæ are killed by the strongest, which is usually the one that hatches first.

Development

The Egg (Plate X, Fig. 1).—The egg is crescent-shaped and translucent. The chorion is smooth and without any reticulations. Under high magnification a brown spot can be seen in the centre of the egg which marks the position of the developing embryo.

First instar (Plate X, Fig. 2).—Freshly hatched larva is opaque white in colour and is composed of a head and thirteen segments. The segmentation is deeply marked. It is crescent-shaped with the anterior end broader than the posterior. Head is hemispherical and broad. Under high magnification mandibles can be seen protruding out from the ventrolateral side of the head. The alimentary canal is prominent and is pale yellow in colour. On the extremity of the caudal segment can be seen eight cuticular spines that are conical in shape (Plate X, Fig. 3). All segments are devoid of setæ and there is no evidence of even a rudimentary respiratory system. Mouth parts are very simple. The mandibles are not so sharp as in the case of *Solenotus*. The tip is golden yellow and the rest colourless. Its length being 0·25 mm.

Second instar.—The second instar larva is somewhat crescent-shaped like that of the previous instar but is a little larger in size. The tracheal system is not seen in this instar.

Third, fourth and fifth instars.—All these instars are crescent-shaped and resemble each other closely except that the size goes on increasing with the age. Their anterior end is broad and rounded while the posterior end is narrow and rounded. Segmentation is quite prominent.

The respiratory system gets well established in the third instar. It consists of two lateral longitudinal trunks, united anteriorly but free posteriorly,

There can be seen seven pairs of spiracles in segments third, sixth, seventh, eighth, ninth, tenth and eleventh. Numerous anastomosing branches pass out to the internal organs.

Mandibles get more heavily chitinized. The body length of the final instar varies from 1·70 to 1·87 mm.

Pupa.—The larva pupates with in its last larval skin, which hardens in due course, but the parasite pupa is always found within the cocoon of the host. The freshly formed puparium is long and oval, and dark brown in colour, but with age becomes black in colour. The developmental stages can be observed easily. A slight constriction appears in between the head and the thorax, and the thorax and the abodomen. The eyes and the other appendages appear gradually. The eyes of the late pupa are quite prominent and scarlet red in colour. The early pupa is dark brown in colour but with age the colour gets changed to dark. The thoracic region develops the conspicuous imaginal wing buds.

The adult (Plate X, Fig. 6).—The adult comes out by breaking open the puparium with the help of its appendages. The freshly emerged *Rhopalotus* is brilliant metallic green.

Respiratory system (Plate X, Fig. 5).—The respiratory system of *Rhopalotus* sp. is well developed in the latter instars but is totally lacking in the first two instars. The intake of oxygen in the first and second instars is from the host blood on which it feeds. In the third instar the open tracheal system makes its appearance. The tracheal system consists of two lateral longitudinal trunks, united anteriorly but free posteriorly. There are seven pairs of spiracles, unlike most other chalcids which have the full component of nine. The spiracles are present on segments third, sixth, seventh, eighth, ninth, tenth and eleventh. The spiracles are attached to the main trunk by means of spiracular stalks, which are longer as compared to that of *Solenotus* sp. The branching of the main tracheal trunk is just similar to that of *Solenotus* sp.

DISCUSSION

Very little work has been done on the parasites of the Dipterous leaf miners. Much of the work in the main relates only to the record of the parasites and to some extent meagre notes on their biology. *Solenotus* sp. as a parasite of the pea leaf miner *Phytomyza atricornis* was first recorded by Ahmad and Gupta (1941) as an endoparasite, which is totally unfounded. It is an ectoparasite which attacks only well-developed larvæ of the leaf miner. Cushman (1926) draws attention to the host preferences of *Sympiecis* sp. which attacks Coleopterous, Dipterous and Hymenopterous leaf miners and

also develops as an external parasite of the egg of *Cimbex*, and he concludes that location rather than the type of the host is the determining factor in selection. This statement of his has no basis as it is always seen that the percentage of parasitisation is dependent on the availability of suitable host stages for parasitisation. The egg of the parasite is like that of any other chalcid. The number of body segments in the first instar larva is generally accepted as thirteen excluding the head. But in the case of *Solenotus*, the thirteenth segment is not clearly demarkated from the fourteenth, and the fourteenth is represented by a pair of papillæ which disappear in the later instars and the fourteenth segment becomes well established. The sensory setæ that are present only in the first instar are characteristically arranged on the dorsum of the larva. The tracheal system of the first instar is open and it consists of five pairs of open spiracles. The general accepted number, however, is only four pairs. Parker and Thompson (1925) in *Solenotus* sp. have shown only four pairs of spiracles.

Rhopalotus sp. is a fine example of what is known as the free living endoparasitic larva defined by Parker and Thompson (1925). The eggs of *Rhopalotus* sp. are laid within the larva or puparia of the host. It is interesting to record a larva of this type as it represents a transitional type between the ecto- and endoparasitic larvæ. This type of larva is strikingly different from conventional endoparasitic type by not possessing a caudal appendage or tail. The first instar stage is apneustic. Such a type has been described by Parker and Thompson (1925) in the case of *Pteromalus puparum* L.

Superparasitism in *Rhopalotus* is very common as one to six eggs are laid within the body of the host. However, only one individual completes its development and emerges as adult. The supernumerary larvæ are killed by the strongest, which is usually the one that has hatched first. The most interesting aspect of this study is the occurrence of multiparasitism among *Rhopalotus* sp. and a braconid, both being endoparasitic. Several hundreds of dissections of the host larvæ have always revealed that the braconid is the one that succumbs. It is surprising to see that the braconid larva which is several times larger and endowed with a pair of powerful sickle-shaped mandibles succumbs to the effect of multiparasitisim. The general belief that some toxic products secreted by one species necessarily kills the other does not hold good in this particular case.

Parker and Thompson (1925), who described the transitional type of endoparasitic larva, have not described the mode of intake of oxygen, when the larva is devoid of both the caudal appendage and the open tracheal system. It is probable that the first and second instars of *Rhopalotus* sp. derive their

oxygen from the host blood, as they feed on them, and by the time the blood becomes scanty the open tracheal system gets established. The braconid gets its oxygen from the host blood through the integument and the caudal appendage only when the host is living. Hence, the moment the host larva which is already parasitised by the braconid is again parasitised by *Rhopalotus*, the braconid immature stages are killed due to lack of oxygen rather than through any other means. One more supporting observation is that the majority of dissections of the host have always revealed the first instar living braconid larvæ and a number of eggs of *Rhopalotus* sp. When the dissections revealed first instar *Rhopalotus* larva the braconid was invariably dead.

The sex ratio of both *Solenotus* and *Rhopalotus* is very high and the percentage of parasitism is also very high. Cohen (1926) recorded 93·3 per cent. parasitism by a braconid on *Phytomyza atriocornis*. The fact that only a negligible per cent. of males are seen in *Solenotus*, it is probable that the chalcid is capable of normal reproduction even without mating.

SUMMARY

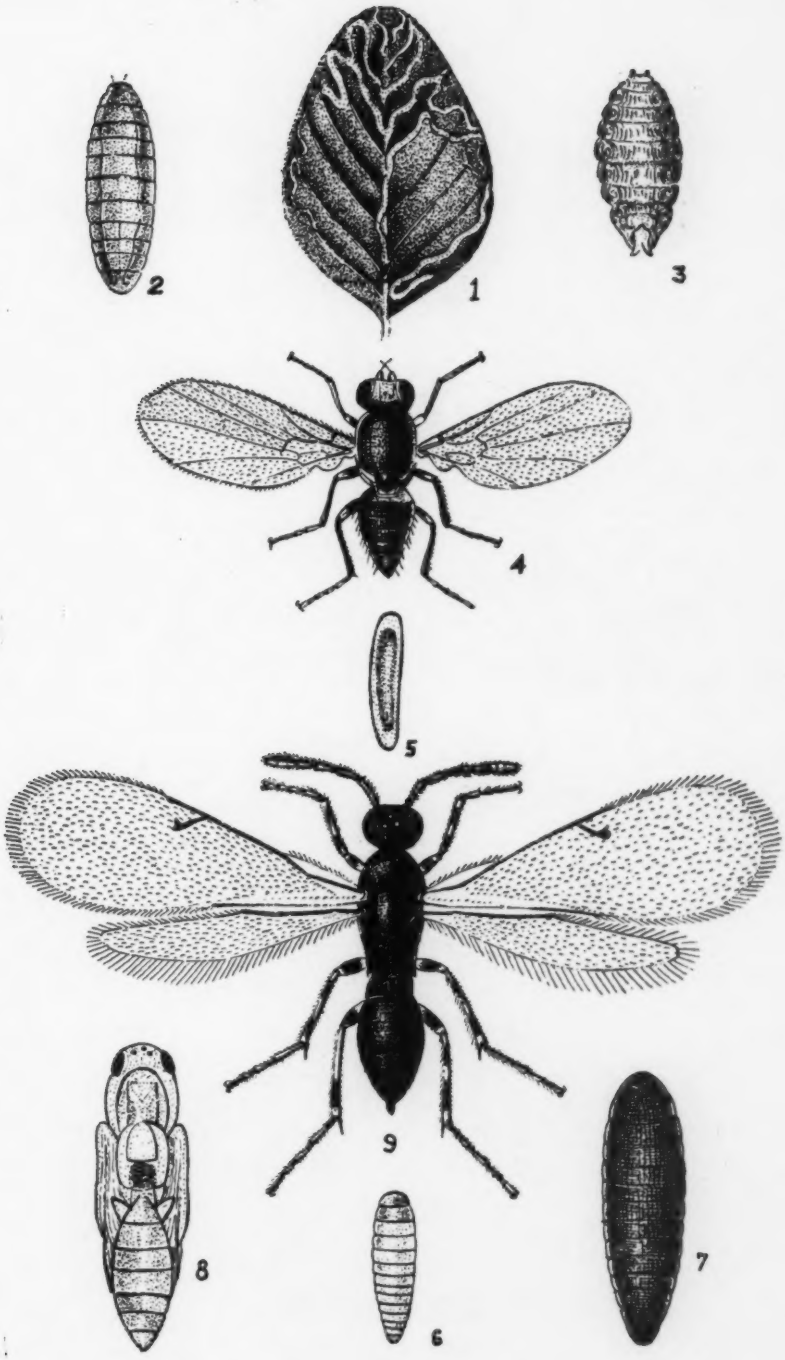
The biology and morphology of *Solenotus* sp. new and *Rhopalotus* sp. new have been studied in detail.

Solenotus sp. is an ectoparasite of the pea leaf miner *Phytomyza atricornis* (Meigan) and *Rhopalotus* sp. is an endoparasite of the same. One more internal parasite of the leaf miner is found to be a braconid. Multiparasitism occurs between the latter two in which *Rhopalotus* species survives and the braconid is killed in the early stages.

The respiratory system differs from other chalcids in having eight pairs of spiracles in *Solenotus* species and seven pairs in *Rhopalotus* species instead of the usual nine pairs. The early stage *Rhopalotus* larva represents a transitional stage between the ecto and endo type of parasitic larva.

The life-histories of *Solenotus* and *Rhopalotus* are more or less similar. In both the cases there are five larval instars. *Solenotus* larvæ feed only on the blood of the host whereas those of *Rhopalotus* feed on the blood as well as on the internal organs. The pupa is naked in the former whereas it is not so in the latter. In both the cases the life-history is completed within ten to fourteen days at room temperature and humidity in the month of March.

Usually, *Rhopalotus* sp. selects chrysalid stage host larva for parasitisation but at times it attacks the last instar larva also. In the former case the development of the parasite is completed while in the latter case the developing grub of the parasite allows the host to form puparia, whereas the parasite itself hibernates till next spring.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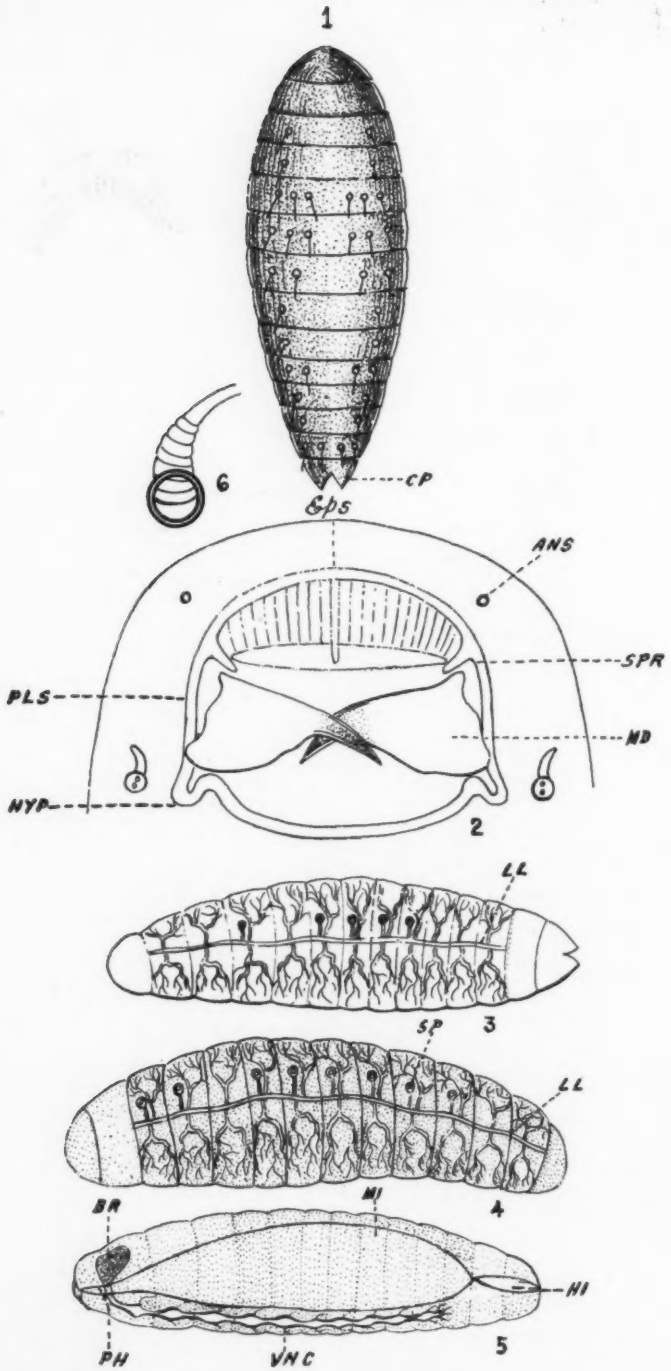
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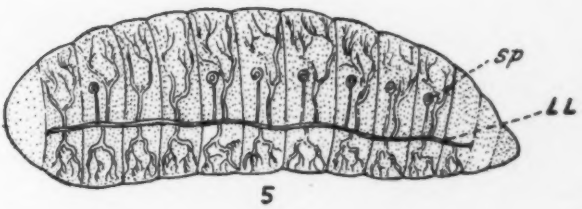

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Explanation of Plates

Plate VIII

Figs. 1–9.—Fig. 1. A pea leaf showing the gallery made by the pest. Fig. 2. The larva of *Phytomyza atricornis* (Meigan). Fig. 3. Pupa of *Phytomyza atricornis*. Fig. 4. Adult fly of *Phytomyza atricornis*. Fig. 5. Egg of *Solenotus* sp. new. Fig. 6. First instar larva of *Solenotus* sp. Fig. 7. Last instar larva of *Solenotus* sp. Fig. 8. Pupa of *Solenotus* sp. Fig. 9. *Solenotus* sp. Adult female.

Plate IX

Figs. 1–6.—Fig. 1. Arrangement of spines on the body of first instar larva of *Solenotus* sp. Fig. 2. Mouth parts and facial rods of the last instar larva of *Solenotus* sp. Fig. 3. Tracheal system of first instar larva of *Solenotus* sp. Fig. 4. Tracheal system of last instar larva of *Solenotus* sp. Fig. 5. Diagrammatic figure showing the digestive and nervous system of *Solenotus* sp. Fig. 6. A spiracle of *Solenotus* sp.

Plate X

Figs. 1–6.—Fig. 1. Egg of *Rhopalotus* sp. n. Fig. 2. First instar larva of *Rhopalotus* sp. Fig. 3. The caudal papillæ of first instar. Fig. 4. Final instar larva of *Rhopalotus* sp. Fig. 5. Tracheal system of *Rhopalotus* sp. Fig. 6. *Rhopalotus* sp. Adult female.

Explanation to Lettering

ANS = Antennal sensilla
CP = Caudal papillæ
EPS = Epistoma
BR = Brain
HYP = Hypostoma
HI = Hind intestine
LB = Labrum
MD = Mandible
MI = Mid intestine
LL = Lateral longitudinal trunk
PH = Pharynx
SP = Spiracle
VNC = Ventral nerve cord

STUDIES WITH SUPERIMPOSED CARBOHYDRATES IN THE FLUE-CURING OF TOBACCO

Part I. Their Effect on the Chemical Changes during Flue-Curing

By A. S. Sastry*

Received April 15, 1956

(Communicated by Dr. S. V. Desai, F.A.Sc.)

Introductoin

The studies enumerated in the present paper are a sequel to the previous observations of the author[1] that the quality of tobacco leaf produced on flue-curing appeared to depend on the carbohydrate content of the green leaf. As this finding is likely to be of far-reaching importance to the agronomist, the plant breeder and to the understanding of the process of flue-curing itself, it is sought to be confirmed by comprehensive experimentation, designed not only to prove this point, but to gain further insight into the role of carbohydrates in the various biochemical processes occurring in the leaf during flue-curing.

Information was sought by inducing differences in carbohydrate concentration in the same leaf by feeding sugar solutions to it and then subjecting it to flue-curing conditions.

The investigations are divided into two parts.

Part I deals with the effect of superimposed carbohydrates on the chemical transformations during flue-curing.

Part II deals with their role in the yellowing of the leaf and the final quality of the cured product.

The first part is presented in this paper and the second will be presented in a subsequent publication.

Experimental

Material and Methods.—The variety employed in these studies was Harrisson's Special, a well established flue-cured variety taken from the experimental plots of the Tobacco Research Station, Guntur, in the year 1947. The method employed to induce differences in the carbohydrate level of the leaves was to culture them in sugar solutions with about 1″ of

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the leaf-base dipping in the solution for the required period and then to subject them to flue-curing conditions.

The experiments described in the following pages had two objectives in view. One was to study on a small scale the effect of artificially induced differences in carbohydrate levels on the chemical transformations during flue-curing and also the yellowing behaviour of the leaf. The second was with a view to conducting a semi-large-scale experiment to evaluate the differences in yellowing by commercial grading analysis (Experiment III).

Towards the first objective two experiments were conducted. In the first, leaf containing large amounts of initial carbohydrate was employed. In the second, leaf with a smaller amount of carbohydrate was used.

The leaf in the first experiment, after removing the initial sample in quadruplicate, was divided into two lots by random sampling. One lot was cultured on water and the other on 5 per cent. glucose solution, for 5 hours (10 A.M. to 3 P.M.) under shade. At the end of the treatment the two lots were again weighed and subdivided into four lots each. These were weighed, strung on separate sticks suitably labelled and subjected to flue-curing. The sticks were removed from the flue-curing barn in duplicate: (1) after culture, (2) at the end of yellowing, (3) at the end of fixing and (4) at the end of curing. The leaf at the end of yellowing and fixing was rapidly divided by professional graders into 3 categories: (1) bright yellow, (2) semi-bright yellow, and (3) dark green to provide a measure of the yellowing behaviour of the leaf, then mixed, weighed and preserved by steaming and drying rapidly in a current of air at 65° C. as described by Loomis and Schull.[2]

The samples were analysed for the same constituents as described by the author in a previous publication.[1] In addition amino nitrogen was determined by Sorenson method.[3] No nitrates could be found in any of the samples.

The volume and concentration of the sugar solution before and after treatment were determined to afford a measure of the amount of sugar taken up by the leaf. Blanks run under the same conditions did not indicate any loss of sugar due to fermentation during the period of culture.

The leaf in the second experiment after preserving the initial sample in quadruplicate was divided into three lots by random sampling and weighed. One lot was given no treatment (allowed to wilt for the period of culture). The second was cultured on water for 24 hours and the third on 5 per cent. sucrose solution for 24 hours (10 A.M. to 10 A.M. next day under shade).

After treatment they were weighed, divided into the required number of sub-lots and proceeded with as before.

Results of chemical analysis are presented in this paper. Results of the yellowing of the leaves in Experiments I and II and grading analysis, in the semi-large-scale experiment (Experiment III), will be presented in Part II.

In these experiments leaves cultured on water always served as controls as compared to those cultured on sugar solutions. However, leaves without any treatment (*i.e.*, wilted for 24 hours) were included in Experiments II and III as these investigations were primarily a study of the effect of the differences in carbohydrate level of the same leaf on its flue-curing behaviour and as wilting would also induce such differences.

Sampling and Analytical Errors.—In order to afford a measure of the sampling and analytical errors that may occur in these studies the initial samples were collected in quadruplicate and analysed for the various constituents as mentioned previously. The mean values and the standard deviations of the various constituents in the initial sample are presented at the appropriate places in Tables II and III. Any variation from the mean value which is more than twice the standard deviation is significant and this is used in the interpretation of the data.

RESULTS

Amount of Sugars Absorbed by the Leaves

By determining the volume and concentration of the sugar solution before and after culture the amount of sugars absorbed by the leaf can be calculated. The data are presented in Table I.

TABLE I

	Experiment I Leaves cultured on 5% Glucose for 5 hours	Experiment II Leaves cultured on 5% Sucrose for 24 hours
Volume of sugar solution absorbed by 100 gm. leaf	12·73 c.c.	35·51 c.c.
Concentration in the beginning of culture	5·0%	5·0%
Concentration at the end of culture ..	4·98%	5·02%
Sugar absorbed by 100 gm. leaf ..	0·64 gm.	1·78 gm.

From the above it may be seen that large amounts of sugars were absorbed by the leaves from sugar solutions, that the amount of sugar absorbed by the leaf increased with the time of culture and that the leaf took up the sugar solution as supplied. Vickery *et al.*[4] also made a similar observation using Rhubarb leaf.

Changes due to Culture

Data on chemical composition of the leaves in Experiments I and II are presented in Tables II and III and the changes due to culture are discussed below:

(1) *Weight of Leaves.*—In both the experiments the leaves gained in weight after culture while the no-treatment leaves in Experiment II lost weight considerably. This was mostly due to the loss of water.

(2) *Carbohydrates.*—In the leaves of Experiment I the level of total carbohydrate was more or less maintained after glucose culture while 0·95 gm. of carbohydrate was lost in water-cultured leaves. However, considerable amounts of organic solids were lost in both the treatments. These were 0·76 gm. in glucose culture as compared to 1·43 gm. in water culture.

In Experiment II there was a gain of 0·41 gm. of carbohydrates in leaves cultured on sucrose while 0·22 gm. and 0·48 gm. of carbohydrates were lost in the water-cultured and no-treatment leaves respectively. Organic solids gained by 0·76 gm. in sucrose-cultured leaves and by 0·29 gm. in water-cultured leaves while 0·79 gm. were lost in no-treatment. The gain observed in water-cultured leaves, however, is not beyond experimental errors.

Thus, it appears that though large quantities of sugars were absorbed by the leaves they did not reappear as carbohydrate increases. The leaves in Experiment I absorbed 0·64 gm. of glucose while there was a decrease of 0·05 gm. of carbohydrates. The leaves in Experiment II absorbed 1·78 gm. of sucrose; but the increase in carbohydrates was only 0·41 gm. The sugars absorbed by the leaves thus appear to have been used up for metabolic purposes. However, the sugar feeding induced differences in the carbohydrate levels. In Experiment I the glucose-treated leaves contained 5·02 gm. total carbohydrates after culture as against 4·12 gm. in water-cultured leaves. In Experiment II the level of total carbohydrates was 2·21 gm., 1·58 gm., and 1·32 gm. in sucrose, water and untreated leaves repectively.

The second point of interest is that while the organic solids decreased considerably in the leaves of Experiment I after culture, they increased

TABLE II

Chemical Changes in the Leaves of Experiment I

(Expressed as grams or milligrams per 100 gram fresh leaf)

		Cultured on 5% glucose				Cultured on water			
	0 hrs.	After culture 5 hrs.	End of yellowing 41 hrs.	End of fixing 56 hrs.	End of curing 150 hrs.	After culture 5 hrs.	End of yellowing 41 hrs.	End of fixing 56 hrs.	End of curing 150 hrs.
1. Weight of leaves	100·00	104·10	66·43	41·76	19·28	107·90	73·28	46·36	19·16
2. Water	80·05	84·80	48·07	24·55	2·12	89·39	55·03	28·98	2·13
3. Total solids	19·95±0·24	19·30	18·36	17·21	17·16	18·51	18·25	17·38	17·03
4. Organic solids	17·17	16·41	15·65	14·44	14·42	15·74	15·43	14·58	14·30
5. Inorganic solids	2·78±0·05	2·89	2·71	2·77	2·74	2·77	2·82	2·80	2·73
6. Reducing sugars as glucose ..	0·67±0·03	0·90	2·08	1·72	1·34	0·63	2·12	2·08	1·36
7. Sucrose as glucose	0·32±0·03	0·37	0·10	0·17	0·60	0·11	0·14	0·05	0·47
8. Total sugars (6 + 7)	0·99±0·04	1·27	2·18	1·89	1·94	0·77	2·26	2·13	1·83
9. Starch as glucose	4·08±0·08	3·75	0·62	0·26	0·24	3·38	0·78	0·38	0·33
10. Total carbohydrate (8 + 9) ..	5·07	5·02	2·80	2·15	2·18	4·15	3·04	2·51	2·16
11. Total nitrogen in milligrams ..	399±19	381	408	387	401	391	417	397	397
12. Protein N ,, ,, ..	274±19	252	197	181	194	256	199	179	201
13. Soluble N ,, ,, ..	125±9	129	211	206	207	135	218	218	196
14. Amino N ,, ,, ..	11±1	10	18	21	22	17	23	25	25
15. Ammonia N ,, ,, ..	2·1±0·05	2·2	2·4	1·8	1·9	2·4	2·2	3·6	1·8
16. Amide N ,, ,, ..	2·1±0·5	2·3	4·5	5·7	5·4	3·2	5·3	7·1	6·1
17. Nicotine N ,, ,, ..	33±0·6	31	32	31	31	32	32	33	31

17. Nicotine N	„	„	..	33±0·6	31	32	31	31	32	32	33	31

TABLE III

Chemical Changes in the Leaves of Experiment II

(Expressed as grams or milligrams per 100 gram fresh leaf)

		No treatment				Cultured on water				Cultured on 5% sucrose			
	0 hrs.	After culture 24 hrs.	End of yellowing 61 hrs.	End of fixing 74 hrs.	End of curing 151 hrs.	After culture 24 hrs.	End of yellowing 61 hrs.	End of fixing 74 hrs.	End of curing 151 hrs.	After culture 24 hrs.	End of yellowing 61 hrs.	End of fixing 74 hrs.	End of curing 151 hrs.
1. Weight of leaves ..	100·00	79·00	56·48	40·56	18·46	112·30	69·26	41·56	19·70	112·70	63·06	45·51	19·63
2. Water ..	80·95	60·61	39·07	23·71	2·00	92·83	50·98	23·72	2·06	92·78	44·55	27·16	1·71
3. Total solids ..	19·05±0·24	18·39	17·41	16·85	16·46	19·47	18·28	17·84	17·64	19·92	18·41	18·35	17·92
4. Organic solids ..	16·14	15·40	14·53	13·93	13·65	16·43	15·39	14·75	14·64	16·90	15·39	15·32	14·98
5. Inorganic solids ..	2·91±0·07	2·99	2·88	2·92	2·81	3·04	2·89	3·09	3·00	3·02	3·02	3·03	2·94
6. Reducing sugars as glucose	0·74±0·03	0·95	0·83	0·62	0·53	0·55	0·65	0·73	0·51	1·13	0·61	0·76	0·86
7. Sucrose as glucose ..	0·00	0·00	0·06	0·20	0·04	0·00	0·24	0·14	0·10	0·00	0·23	0·11	0·11
8. Total sugars as glucose (6+7)	0·74±0·03	0·95	0·89	0·82	0·57	0·55	0·89	0·87	0·61	1·13	0·84	0·87	0·97
9. Starch as glucose ..	1·06±0·04	0·37	0·07	0·05	0·01	1·03	0·11	0·04	0·01	1·08	0·11	0·05	0·03
10. Total carbohydrate (8+9)	1·80	1·32	0·96	0·87	0·58	1·58	1·00	0·91	0·56	2·21	0·95	0·92	1·00
11. Total nitrogen (in mg.)	488±17	499	489	504	497	504	516	501	513	502	517	497	513
12. Protein N „	317±14	266	192	180	207	320	215	214	221	308	236	205	218
13. Soluble N „	171±4	233	297	324	290	184	300	287	292	194	281	292	295
14. Amino N „	15±1	29	53	54	44	19	52	48	42	17	52	56	47
15. Ammonia N „	3±0·4	8	20	25	15	8	21	21	13	7	16	20	17
16. Amide N „	5±0·9	16	29	32	33	1·5	27	28	33	−1	18	28	35
17. Nicotine N „	47±1·5	48	48	49	47	47	49	50	49	49	50	48	49

somewhat in the leaves of Experiment II. This may be because of differences in the photosynthetic activity of the two sets of leaves.

Several workers observed a decline in photosynthetic activity with the accumulation of assimilation products.[5, 6, 7] The inhibiting effect of carbohydrates explains the low photosynthetic activity of the leaves of Experiment I as compared with those of Experiment II.

(3) *Nitrogenous Constituents.*—The data show that there were no significant changes in total nitrogen and protein nitrogen of the leaves in both the experiments. The amino nitrogen appeared to slightly increase in the water-treated leaves in both the experiments, while the changes in this constituent in sugar-treated leaves were not significant. The method used in the determination of amino nitrogen estimates the amino nitrogen of not only the amino acids but also soluble peptides. The increase in amino nitrogen, in the absence of hydrolysis of proteins, suggests that the increase in this constituent in water-treated leaves was probably due to the breakdown of peptides into simpler amino acids. There were no significant changes in the other nitrogenous constituents due to culture in Experiment I, while in Experiment II, small quantities of ammonia accumulated in both the treatments.

In the no-treatment leaves of Experiment II, on the other hand, significant amounts of proteins were hydrolysed and soluble nitrogenous constituents increased appreciably, particularly amino nitrogen, ammonia and amides.

Chemical Changes during Flue-Curing of the Cultured Leaves

The effect of culture in sugar solutions on the chemical composition of the leaves has been considered in the foregoing pages and it has been shown that sugar feeding induced differences in the carbohydrate levels of the leaves, though all the sugar absorbed by the leaves had not reappeared as carbohydrates, partly due to losses in respiration and partly due to conversion into other metabolic products by the life activities of the leaf-cells. The effect of these differences in carbohydrate levels on chemical transformations in the leaves during flue-curing will be considered in the following pages, and will be dealt with under the heads (1) water and solids, (2) carbohydrate constituents, and (3) nitrogenous constituents.

Water and Solids.—From the data in Tables II and III it appears that the loss of weight of leaves was mostly due to water. In Experiment I while the glucose-cultured leaves lost more weight during yellowing (37·1 gm.) than water-cultured leaves (34·6 gm.) the reverse was true during fixing (glucose-

cultured leaves lost 24·6 gm. as against 26·9 gm. for water-cultured leaves). Similar trends were observed in Experiment II also. These results appear to indicate that the conditions in the barn were not quite uniform, though the greatest care was taken in stringing the leaves and in placing the sticks at equal distances from one another.

It can also be seen that the no-treatment leaves in Experiment II lost weight more slowly during yellowing than the cultured leaves. This probably was due to the type of water that was involved. In the former it might be the cellular water, while in the latter the intra-cellular water.

Varying amounts of total solids were lost in the two sets of leaves. The inorganic constituents more or less remained unchanged thus throwing the entire loss on organic solids.

In Experiment I, 0·76 gm. of organic solids were lost during the yellowing of glucose-cultured leaves as against 0·31 gm. in water-cultured leaves. In Experiment II, 1·51 gm., 1·04 gm. and 0·87 gm. were lost during yellowing in sucrose-culture, water-culture and no-treatment leaves respectively. It is thus obvious that more organic solids were lost from sugar-cultured leaves as compared with controls in both the experiments. The loss of organic solids proceeded during fixing period and at a much smaller rate during drying period also and as during these stages the leaf cells rapidly disintegrate the significance of this loss cannot be assessed.

Carbohydrate Constituents.—The leaves in Experiment I contained 3 times as much carbohydrate as those in Experiment II and in both these sets of leaves significant differences in carbohydrate levels were attained after culture.

During yellowing starch hydrolysis proceeded with equal rapidity both in water-treated and sugar-treated leaf in both the experiments. While reducing sugars and total sugars increased in the leaves of Experiment I, in Experiment II the changes in these constituents were not large. This was perhaps because the leaves in Experiment II contained smaller amounts of carbohydrates to start with and in consequence not only the starch was hydrolysed but also the sugars produced were used up in respiratory processes. Even in the leaves of Experiment I the increase in sugars was not enough to compensate for the loss in starch, as was evident from the decrease in total carbohydrates and the difference must have been used up in the respiratory and other cell processes.

Starch hydrolysis proceeded at a much lower rate during fixing and during drying it was insignificant in both the experiments.

Reducing sugars which attained a maximum at the end of yellowing in Experiment I decreased slightly during fixing. The evidence points to slight losses of sugars during fixing in glucose-treated leaves while in the water-cultured leaves large losses of sugars occurred during drying. In the leaves of Experiment II, slight losses of sugars occurred during drying in the no-treatment and water-cultured leaves, while no such losses occurred in the sugar-cultured leaves. This appears to suggest that in trying to cure differently treated leaves in the same barn, conditions ideal for all types of leaves could not be maintained. Similar decreases in sugars in the high-temperature conditions had been reported by Askew *et al.*[8] also.

Large quantities of sucrose were synthesised during the drying stage in both water-cultured and glucose-cultured leaves in Experiment I. This was not important in the leaves of Experiment II.

Nitrogenous Constituents.—The changes in total nitrogen of the leaves were not significant in any of the treatments in both the experiments. This is in conformity with the previous findings of the author[1] and also of Askew *et al.*[8]

Protein hydrolysis commenced immediately on flue-curing the leaves and the rate of hydrolysis was the greatest during yellowing. During the later stages of curing the insoluble nitrogen appeared to increase somewhat, due probably to the secondary reactions of simpler nitrogenous constituents with other compounds of the leaf. Such a phenomenon was noticed by Askew *et al.*[8] also.

As proteolysis took place both soluble nitrogen and amino nitrogen increased in all the treatments during yellowing. In the leaves of Experiment II the amino nitrogen formed was thrice as much as in those of Experiment I.

Ammonia nitrogen did not vary much in the leaves of Experiment I while in those of Experiment II a considerable quantity of ammonia was produced in all the treatments during yellowing. In the sucrose-cultured leaves, it was a little less than in the others.

Amide formation was 4 to 10 times as much in the leaves of Experiment II as in those of Experiment I. In the leaves of Experiment I the amount of amides formed during yellowing was not significantly different in sugar-cultured leaves from that in water-cultured leaves. In Experiment II, on the other hand, amide formation was a little less in sugar-cultured leaves than in others.

Discussion

The present investigations provide a good opportunity to test whether carbohydrates have any sparing action on protein hydrolysis in starving leaves. The data tend to show that in leaves cultured in water or sugar solutions in both the experiments, proteins remained intact. Vickery *et al.*[9] found that in Connecticut shade-grown tobacco protein hydrolysis proceeded at approximately the same rate for about 100 hours, both in light and darkness and subsequently the rate of hydrolysis in illuminated leaves diminished. The illuminated leaves meanwhile acquired a large store of organic substances more than half of which were present as soluble reducing sugars. The leaf of Vickery *et al.*[9] contained only 0·04 per cent. starch and 0·25 per cent. total sugar on green basis and after culture in light for 143 hours (figures at the end of 100 hours had not been given), it accumulated 0·2 per cent. starch and 1·6 per cent. total sugars, a total of 1·8 per cent. total carbohydrates which was enough to put a check on protein degradation. The leaf in Experiment I contained 5 per cent. total carbohydrates to start with and that in Experiment II about 1·8 per cent. which was almost the same as what Vickery's leaf acquired after 143 hours culture in light. Thus, the high carbohydrate content of the present set of leaves appears to have prevented protein digestion during culture.

It is of great scientific interest to see whether carbohydrates added through sugar culture have the same effect on the chemical transformations during flue-curing as those *in situ*.

Data of Curings B and D, culled from the author's previous work,[1] provide good material to illustrate the differences in chemical transformations brought about by *in situ* differences in carbohydrate levels. These two curings have been chosen, as the leaves used in them contain different amounts of carbohydrates with almost the same amount of nitrogen. The data are presented in Table IV.

Table IV

Data from Curings B and D of Sastry (1953)

	Curing B	Curing D
Total carbohydrates percentage green leaf	5·13	3·45
Total nitrogen percentage green leaf	0·434	0·423
Loss in carbohydrates as percentage loss in organic solids during yellowing	85·5	64
Ammonia nitrogen gain during yellowing	1·0	5·0
Amide nitrogen gain during yellowing	0·5	56·0

The data are self-explanatory. In the leaves of Curing B which contained about 5 per cent. initial carbohydrates, carbohydrates could meet 85 per cent. of the respiratory demands of the leaf. In the leaves of Curing D which contained much less carbohydrates only 64 per cent. of the respiratory losses were met by carbohydrates and more of other constituents were drawn on for respiration. The large increases in amides and ammonia in these leaves compared to those in Curing B suggest that these may be of protein origin.

Reverting back to the present investigations, the losses in carbohydrates and organic solids during yellowing are presented in Table V, and the nitrogen balance data in Tables VI and VII.

TABLE V

Loss in Organic Sotids and Carbohydrates during Yellowing Expressed as grams per 100 gm. Fresh Leaf

	Experiment I		Experiment II		No treatment
	5% glucose	Water	5% sucrose	Water	
Org. solids	0·76	0·31	1·51	1·04	0·87
Carbohydrates	2·22	1·11	1·26	0·58	0·36
Carbohydrates % Org. Solids	292·1	358·0	83·45	55·77	41·38

TABLE VI

Nitrogen Balance at the End of Yellowing in the Leaves of Experiment I

(Expressed as milligrams per 100 gm. fresh leaf)

	Water culture	Glucose culture
1. Protein N hydrolysed	75	77
2. Amino N gain	12	7
3. Ammonia gain	0·1	0·3
4. Amide N gain × 2	6·4	4·8
5. Amino N actually produced from proteins	18·5	12·1

TABLE VII

Nitrogen Balance at the End of Yellowing in the Leaves of Experiment II

(Expressed as milligrams per 100 gm. fresh leaf)

	No treatment	Water culture	Sucrose culture
1. Protein N hydrolysed	125	102	81
2. Amino N gain	38	37	37
3. Ammonia N gain	17	18	13
4. Amide N gain × 2	48	44	26
5. Amino N actually produced from proteins	103	99	76

In the leaves of Experiment I the losses in carbohydrates were more than enough to meet the respiratory demands of the leaf during yellowing. Differences in amino nitrogen, ammonia and amides formed were not large in water- and glucose-cultured leaves.

In the leaves of Experiment II which contained much smaller amounts of carbohydrates, than in those of Experiment I, other constituents were drawn on for respiratory purposes in all the treatments. Consequently more proteins were hydrolysed, and greater amounts of ammonia, amides and amino nitrogen were produced in these leaves as compared with those in Experiment I (Tables VI and VII).

Taking the sugar- and water-cultured leaves of Experiment II alone into consideration the loss in carbohydrates could meet 83 per cent. of the respiratory losses in the former, while they contributed to only 56 per cent. of the respiratory losses in the latter. The amount of ammonia and amides formed also were much less in the former than in the latter.

These results suggest that carbohydrates from extraneous sources play the same role as those *in situ*.

SUMMARY

The previous work of the author[1] indicated that the quality of leaf produced on flue-curing was dependent on the carbohydrate content of the green leaf. Experiments were therefore devised to artificially induce differences in the carbohydrate content of the same leaf by sugar culture and

study their effect on chemical transformations during flue-curing and the quality obtained on curing.

Two experiments, one with high-carbohydrate leaves and the other with low-carbohydrate leaves, were conducted. In this part chemical transformations in the cultured leaves during flue-curing have been considered. The findings are briefly as follow:

(1) The leaf absorbed a large quantity of sugars during sugar culture. However, all the sugar absorbed was not present as such in the leaf. This was due to metabolic conversions and respiratory losses in the leaf. The photosynthesising ability of the leaves was found to play a part too. Due to these, the sugar-fed leaves contained more carbohydrates than controls.

(2) Protein remained intact in these leaves during culture. Probably the high-carbohydrate content of these leaves, as compared to those in literature, exercised a sparing action.

(3) The losses in carbohydrates were more than enough to meet the respiratory demands in the leaves of Experiment I, which contained a high level of carbohydrates. The quantities of ammonia, amides and amino nitrogen formed were not much. In the leaves of Experiment II, on the other hand, constituents other than carbohydrates were increasingly drawn on for respiration in the order sugar culture, water culture and no-treatment. Accumulation of large quantities of ammonia, amides and amino nitrogen showed that they might be of protein origin.

(4) Comparison with the high- and low-carbohydrate leaves of the author's previous work (*loc. cit.*) showed that the role of added carbohydrates was similar to those *in situ*.

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STUDIES WITH SUPERIMPOSED CARBOHYDRATES IN THE FLUE-CURING OF TOBACCO

Part II. Their Effect on the Yellowing of the Leaf during Flue-Curing and its Final Quality

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Introduction

The present series of investigations have been undertaken to confirm the previous findings of the author[1] that the carbohydrate content of the green leaf appeared to determine the quality produced on flue-curing. Differences in the carbohydrate level of the same leaf were induced by culturing the leaves in sugar solutions. The role of these superimposed carbohydrates in the chemical transformations during flue-curing have been dealt with in Part I. It has been shown therein that carbohydrates added or *in situ* bring about similar chemical changes. In the present section it is proposed to deal with the effect of these on the yellowing of the leaf during flue-curing and on the quality of leaf produced on curing.

Material and Methods

The methods of sugar-culture and sampling have been dealt with in Part I. The yellowing of the leaf was studied by removing suitable samples of the leaf from the flue-curing barn at the end of yellowing and fixing in the Experiments 1 and 2 referred to in Part I, and by dividing it rapidly into three categories (1) bright yellow; (2) semi-bright and sponged; and (3) dark green by experienced graders.

Also a few experiments were conducted to record the course of visual changes in the yellowing of sugar and water-cultured leaves. A number of leaves were cut along the mid-rib, one set of half leaves were cultured on water and the other on 5 per cent. sugar solution for 21 hours. The half leaves were then subjected to flue-curing conditions in the laboratory and the course of visual changes during yellowing recorded as accurately as possible.

For studying the influence of carbohydrates on quality, the leaves were divided into three large lots of 30 lb. each by random sampling and were

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TABLE I

Visual changes during yellowing of leaves cultured on 5 per cent. sucrose solution and water

Leaf No.	After 21 hrs. culture		After 6½ hrs. yellowing		After 13½ hrs. yellowing		After 21 hrs. yellowing	
	Sugar	Water	Sugar	Water	Sugar	Water	Sugar	Water
1	Light green	Dark green	Light yellowish green throughout	Dark green slight yellow at tips	Greenish yellow; uniform	Margins yellowing; near mid-rib and base green	Uniform yellow with slight green tint	Uniform yellow with greater green tint than sugar-treated
2	Light yellowish green throughout	Light green margins yellower than the rest	Yellowish with slight green, uniform	Yellowish; more green at base; tips and margins slight yellow	Yellow with slight green throughout	Complete yellow; slight green near base and mid-rib	Same as above	Yellow; sponging at tips
3	Same as above	Darker green tips yellower than the rest	Same as above	Green at base; tips yellow	Same as above	Upper portions yellow; base slight green	Same as above	Same as sugur treated but slight green near base and mid-rib
4	Light green; upper half yellow green	Light medium green; upper portion less yellow	Greenish yellow	Greenish yellow; base more green	Same as above	Same as above	Same as above sponging at tips	More yellow than sugar treated. Brownish throughout
5	Uniform light green	Darker green; middle portion yellow	Light medium green; uniform	Green; middle and tips yellowing	Slight green with yellow tint	Middle portions yellow; rest green	Greenish yellow	Greenish yellow, green more

subjected to flue-curing (1) after no-treatment for 24 hours, (2) after culture on water for 24 hours, and (3) after culture on 5 per cent. cane-sugar solution for 24 hours. After curing and bulking in the normal way, they were graded by professional graders according to commercial grading procedures. It is necessary to state here that the graders did not know the significance of the work they were doing and therefore could not be biased in their judgment.

RESULTS

Yellowing behaviour of the leaves

Data pertaining to the course of visual changes during yellowing of sugar and water-cultured half leaves are presented in Table I.

Although such information has been collected for a number of leaves, data of only five leaves have been presented in Table I, as all the others showed similar trends. These data clearly show that yellowing in sugar-cultured halves occurs over the entire surface, while in water-cultured leaves it is in patches.

The data relating to the yellowing of the leaf in Experiments I and II are presented in Tables II and III.

TABLE II

Yellowing of the leaf during flue-curing (Experiment I)

(Results expressed as grams per 100 grams fresh leaf)

	At the end of yellowing		At the end of fixing	
	Water cultured leaves%	5% glucose cultured leaves	Water cultured leaves%	5% glucose cultured leaves%
Bright yellow	32·36	38·88	37·48	51·32
Semi-bright and sponged ..	38·24	33·34	42·06	35·35
Dark green	29·40	27·78	20·46	13·33

The results clearly show that sugar-cultured leaves yellow better than controls in both the experiments. The decrease in bright yellow leaves at the end of fixing in all the treatments in Experiment II suggests that the leaves have been kept too long in the barn before fixing while trying to yellow the untreated leaves.

TABLE III

Yellowing of leaf during flue-curing (Experiment II)

(Results expressed as grams per 100 grams fresh leaf)

	At the end of yellowing			At the end of fixing		
	No treatment	Water cultured leaves	Sucrose cultured leaves	No treatment	Water cultured leaves	Sucrose cultured leaves
Bright yellow	21·34	33·51	53·22	3·14	25·17	33·42
Semi-bright and sponged ..	57·37	45·03	41·64	79·68	74·83	66·58
Dark green	21·29	21·46	5·14	17·18	nil	nil

The effect of carbohydrates on the quality of the cured leaf

The grading analysis of the cured leaf obtained in the semi-large scale experiment (Experiment III) is presented in Table IV.

TABLE IV

(*Experiment III*)

Grading analysis of the cured leaf in the semi-large scale experiment

	No treatment	Cultured on water for 24 hours	Cultured on 5% sugar for 24 hours
Grade 1 ..	0·00	0·00	0·00
„ 2 ..	0·00	0·00	0·00
„ 3 ..	3·52	7·69	12·01
„ 4 ..	1·32	2·14	3·00
„ 5 ..	20·26	14·10	26·17
„ 6 ..	18·50	13·68	23·17
„ 7 ..	44·04	38·89	20·18
Light green ..	2·64	5·55	2·15
Dark green ..	7·49	14·96	11·16
Scrap ..	2·20	2·90	2·15

The above data clearly indicate that a greater percentage of higher grades (grades 3 and 4 in this case) are produced in the leaves cultured in sugar solution as compared with those in no-treatment and water-culture.

DISCUSSION

The foregoing data strongly suggest that carbohydrates in some way bring about better and more uniform yellowing of tobacco leaves during flue-curing. The question that naturally arises is " What is the role of carbohydrates in yellowing ? " To answer this question it is necessary to briefly review the literature on yellowing of leaves.

The yellow colour of the leaves is due to carotenoids and flavones. Even in the green leaves chlorophyll is regularly accompanied in the plastids by the yellow carotenoids, whose presence remains concealed because of the more abundant green chlorophyll. During yellowing the chlorophyll changes into the chlorophillides *a* and *b* and phytol, all of which are colourless, and the yellow colour becomes visible.

In this connection, the studies of Michael[2] with nasturtium leaves (*Tropæolum majus*) are of exceptional interest. Leaves of this particular plant have been employed as they yellow readily and progressively from the point of convergence of the leaf ribs, with such a sharp boundary between the green and yellow portions, that the extent of yellowing could be measured with a planimeter. Michael observed a profound difference between the yellowing of the leaves kept in the dark and light. In the light, the yellowing instead of spreading out evenly from the leaf base as in the dark, took place slowly over the entire surface of the leaf, which gradually changed from deep-green to pale-green, yellow-green and finally pure yellow. He explained this uniform yellowing of the leaves in the light, as due to renewed synthesis of proteins, from their fission products under the action of light, so that there was no place of preferential proteolysis, as at the base in the dark. His experiments, further showed, that there was a constant ratio between chlorophyll and protein even when portein decomposition is hastened or retarded. The presence of protein decomposition products was of importance too, where there was no translocation, yellowing was retarded.

Chibnall and Grover[3] showed that the same ratio existed between chloroplastic and cytoplasmic proteins in the leaves of runner bean both before and after four days in the dark. Hence both the fractions can be used.

Jordan and Chibnall[4] found that the breakdown of ptotein was accompanied by disappearance not only of chlorophyll but of carotene , xantho-

phyll, glycerides and phosphatides also. In yellowing a general breakdown of the protoplasm appears to occur. Fagan and Aston[5] corroborated this.

Several workers[6–9] hold the view that chlorophyll is held by chemical forces on proteinaceous matter, degradation of proteins being accompanied by equivalent disappearance of chlorophyll.

In the modern view[10–13] concomitant synthesis and breakdown of nitrogen compounds—and chlorophyll is a nitrogen compound—is regarded as a characteristic of life.

To the author, it appears, that concomitant synthesis and breakdown of proteins and other nitrogen compounds is a characteristic of yellowing leaves also, as yellowing is a life-process, the disappearance of proteins observed in these leaves being only due to the differences in the rates of synthesis and proteolysis. In such a scheme, what is the role of carbohydrates ?

The mechanism of protein synthesis in plants is but incompletely understood. Several workers[14–19] hold the view that proteins and amino acids are in equilibrium with one another and that protein synthesis takes place by step-by-step condensation of amino acids. The alternate view that protein synthesis and proteolysis are two distinct processes, not merely aspects of a reversible system, is supported by Gregory and Sen[20] and Steward and co-workers.[21–25]

Whatever be the route by which proteins are synthesised in the plants, carbohydrates play an important role in its synthesis. If the protein synthesis occurs through condensation of amino acids then carbohydrates, which are linked with amino-acids through α-keto acids, limit according to Wood[22] amino-acid formation in starvation experiments where carbohydrates and proteins decrease simultaneously. In the alternate view, advanced by Steward *et al.*,[27] protein arises directly from sugar (or sugar derivatives) and ammonia, rather than that the individual amino acids are separately synthesized and then condense *via* peptides to proteins.

Thus the role of carbohydrates in more uniform yellowing of leaves appears to be in facilitating the reactions of synthesis of nitrogen compounds and thus causing a more uniform degradation of protein and along with it chlorophyll in all the cells of the leaf. The observations of Michael[2] lend support to this view. He found that nasturtium leaves yellow more uniformly in light and ascribed this to renewed synthesis of proteins from their fission-products under the action of light, so that there was no place of preferential proteolysis as at the base in the dark. To the author it appears, that this

may as well be due to the carbohydrates produced in the light. The more uniform yellowing of sugar-cultured half-leaves (Table III) lends support to this view.

If this were the role of carbohydrates in producing a more uniform yellow colour of the leaf, then it is not only the amount of carbohydrates but also their mobility, which is dependent on the amount of water in the leaves, which is of importance.

The data presented in Tables II and III may be examined from this angle. The carbohydrate and water content in the leaves of Experiments I and II are presented in Tables V and VI.

TABLE V

Leaves in Experiment I

(Results expressed as grams per 100 grams fresh leaf)

	Cultured on water	Cultured on 5% glucose
Carbohydrates after culture ..	4·15	5·02
Water after culture	89·39	84·80

TABLE VI

Leaves in Experiment II

(Results expressed as grams per 100 grams fresh leaf)

	No treatment	Cultured on water	Cultured on 5% sucrose
Carbohydrates after culture ..	1·32	1·58	2·21
Water after culture	60·61	92·83	92·78

The leaves cultured on sugar solutions contained more carbohydrates than those on water in both the experiments and they to a large extent account for the better yellowing of the sugar-fed leaves as compared with controls. In the leaves of Experiment II, however, the difference in the carbohydrate levels of no-treatment and water-cultured leaves does not appear to be great enough to account for the differences in yellowing. However, the low water

content or low mobility of carbohydrates appear to explain the poor yellowing of no-treatment leaves as compared with those cultured on water.

Summary

1. The yellowing of the leaf cultured on sugar solutions was found to be better than that in controls.

2. This was found to be due to the higher carbohydrate content of the sugar-fed leaves, as compared to controls.

3. After briefly reviewing the literature on yellowing, a hypothesis on the role of carbohydrates has been suggested.

4. The water content of the leaves was found to play a part too and this was explained as due to the mobility of carbohydrates.

5. The better grading analysis of sugar-fed leaves as compared to controls in a semi-large scale experiment was in line with the yellowing behaviour of the sugar-fed leaves.

Acknowledgment

The author desires to acknowledge with thanks the help and guidance of Dr. S. V. Desai, who was in charge of the Division of Chemistry, Indian Agricultural Research Institute, at the time these investigations were carried out.

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STRUCTURES REVEALED BY DARK GROUND ILLUMINATION IN LIVING YEAST

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Introduction

The nucleus in the living yeast shows a range of variation in its structure in cells from a 96–120-hr. wort culture at 22–25° C. of a strain of *Saccharomyces cerevisiæ* (Royan and Subramaniam, 1956). After offering proof that the staining reactions of the body surmised to be the nucleus are identical with that of the nucleus described in yeast by the majority of yeast cytologists, the variations in its structure in the living state was illustrated with photomicrographs taken under the phase contrast type of illumination (Royan, 1956).

It was shown that iodine-formol-acetic solution gives a life-like preservation except for the slight reduction in the overall size of the cell. Hydrolysing the smears in N HCl for 8–10 min. at 60 °C. and staining with iron hæmatoxylin by the long method of Heidenhain reveals only the structures seen in the living nucleus. When a living nucleus appears homogeneous no further details could be revealed by staining. If, on the other hand, it contains formed structures, staining reveals the bodies as well as the nuclear membrane (Royan, 1956).

If the nucleus is visible in a living cell with ordinary illumination it could be located under the ultra-microscope also (Photos 4, 5 and 6, Royan and Subramaniam, 1956). Evidence was adduced for the first time that a nuclear membrane could be seen under dark ground illumination in favourable examples.

Guilliermond (1941) reported that in some yeast cells the vacuole has a luminous contour. An investigation in this laboratory (Aswatha Narayana, 1956) showed that with the increasing age of the culture more and more cells exhibited luminous contours to their vacuoles. It was presumed that such luminous borders signify the presence of a well-formed membrane separating the vacuole from the cytoplasm.

At certain stages therefore the nucleus and the vacuole are delimited from the cytoplasm and each other by well-formed membranes (Royan and

Subramaniam, 1956). From observations with the phase contrast microscope, supplemented by stained preparations, it was concluded that often formed structures are present inside the nucleus (Royan, 1956). Objections have been voiced (Mundkur, 1954) regarding the real existence of the structures revealed by fixation. Wolman (1955) remarks: "There are no good universal fixing agents any more than there are universally good stains." "Different appearances of the same structures studied by different methods do not necessarily mean that one of the methods elicits the formation of artifacts" (p. 99).

When a structure is seen in a living cell it is easy to evaluate its reactions to fixatives and judge accurately the changes produced. In yeasts, such an evaluation becomes imperative since only in some strains of yeasts and that under specified conditions could the nucleus be observed in the living state. Generally the nucleus is invisible and hence most of the discussions on the relative values of the fixatives employed to elucidate its structure and behaviour can at best be considered only academic.

In the above context the desirability of studying the living nucleus (Chambers, 1925; Hughes, 1952) by varying methods of approach would commend itself. The observations under dark ground illumination were carried out to elucidate, if possible, answers to the following questions: (1) Is the nucleus inside or outside the vacuole? (2) Are there formed structures inside the nucleus?

MATERIAL AND METHODS

Considerable care had to be exercised in the selection of material to illustrate the observations. Though the nucleus could be seen in 50–60% of the cells in 96–120-hr. wort cultures at 22-25 °C. of a strain of *Saccharomyces cerevisiæ*, the cytoplasm often contains granules which mask the clarity of the structures in photos taken under phase contrast as well as dark ground illumination. This necessitated selection of cells in which the granules were absent or were away from the structures chosen for illustration. Most of the cells in the culture were not budding.

Apart from the reflection of the cytoplasmic granules blurring the structure of the nucleus and vacuole, close proximity of the cells themselves interfered with the correct delineation of the cell organelles. Therefore a very thin suspension of cells in the medium in which they had grown was mounted on a clean slide under a coverslip. It was discovered that to get good photos of the luminous contours of the vacuoles, there should be no flattening of the cells. On the other hand, the structural details of the nucleus are clear

only when the cells are slightly flattened. These desiderata could be achieved by the use of filter-paper strips on either side of the coverslip.

The equipment in our possession did not enable photographing the same cell under phase contrast as well as dark ground illumination. A green filter was used to improve the clarity of the negatives obtained with the ultramicroscope. The photographs were taken on Kodak 35 mm. 'Microfile' film with a Leica attachment. They were enlarged to a magnification of ca. ×2,500.

Observations

The orientation of the nucleus as observed under the phase contrast microscope is illustrated in Photos 1, 2 and 3 A. When two vacuoles are present the nucleus lies in the cytoplasmic band between the two (Photos 3 B, 4 and 5). There is no canal connecting the two vacuoles. Attention is invited to the variations in the structure of the nucleus in the six cells. The nuclear membrane can be seen in favourable instances (Photos 2 and 3 B), but often the nuclear matrix adjacent to the membrane appears denser (Photos 1, 3 A and 4). Well-formed structures when present exhibit varying dispositions (Photos 2, 3 A, B, and 4) inside the nucleus.

Keeping in view the differing dispositions of the nucleus as well as the structural variations observed in it under phase contrast, an examination under dark ground illumination becomes interesting. The luminous contour of the vacuole is striking enough to suggest that it is a well formed membrane (Photos 6, 7 and 8). When the vacuolar membrane is critically focussed, the nucleus goes out of focus. But the position of the nucleus is easy to recognize (*NA*, Photos 6, 7 and 8). In bivacuolate cells each vacuole has a luminous contour (*VM*) and the nucleus (*N*) lies between them (Photo 7). Well-formed membranes delimit each vacuole from the cytoplasm as well as the nucleus.

It was indicated earlier that the cells should be slightly flattened to get the nuclear details. But when some of the medium is removed by filter-paper strips and the cells are flattened by the pressure of the coverslip, it becomes rather difficult to include the nuclear details as well as the vacuolar membrane in the same photograph. In instances where both are seen (Photo 9), the disposition of the nucleus in relation to the vacuole is found to be similar to that in phase contrast micrographs (compare Photo 9 with Photo 1). When the nucleus appears structureless in phase contrast, it could be seen under dark ground as an area having a luminous contour. Such a condition is illustrated by Photos 10 and 11 of the same cell taken at

two differing foci. The luminous contour is slightly fuzzy unlike the clean-cut appearance of the vacuolar membrane (Photos 6, 7 and 8). A pair of granules may be seen lying outside the nucleus (Photos 10 and 11).

Unlike in Photos 10 and 11, the nucleus of the cell in Photo 12 has a well formed membrane which encloses a grain inside. The periphery of the grain appears as a luminous circle in the plane at which the photo was taken. In other foci it merges with the luminous cell wall. Sometimes the area of the nucleus can be recognized only by the granules occurring at its periphery (Photo 13). A nuclear membrane as such being not visible it is impossible to determine whether these grains are inside or outside the nuclear membrane. Such configurations are not rare (Photo 14).

Cells in which differing regions of the nuclear boundary appear thick are quite common (Photos 15, 16, 17, 18, 19, 20 and 21). As a consequence their shape may be said to resemble roughly that of a horse-shoe. The open ends, however, are joined together by the thin nuclear membrane. The thickness of the two limbs may (Photo 16) or may not (Photos 17, 18, 19, 20 and 21) be uniform. The orientation of the open end of the horse-shoe with reference to the vacuole shows considerable variation (Photos 9, 16, 17, 18, 19, 20 and 21). Owing to the reflection from the cell wall it is difficult to judge whether it could be near the wall also (Photos 22 and 23).

Nuclei having such a configuration may (Photos 17, 18, 19 and 20) or may not contain light reflecting structures inside. Presence of bodies inside the nucleus are revealed in Photos 17, 18, 19 and 20 of two cells taken at two different foci. A comparison with phase photographs 1–5 would emphasize the similarity of the structures seen under the two differing types of illumination.

The existence of a well-defined vacuolar membrane near the nuclear area (Photos 6, 7 and 8) and the presence of a thick nuclear boundary where it abuts on the vacuole (Photos 22 and 23) leads one to the belief that the vacuole has no connection with the nuclear matrix.

DISCUSSION

To the question whether the nucleus is inside (Lindegren, Williams and McClary, 1956) or outside the vacuole (Guilliermond, 1920; Subramaniam 1946, 1952; Lietz, 1951; Mundkur, 1954) a definite answer seems possible. The vacuole and the nucleus are separate structures (Phase Photos 1–5; Dark ground Photos 9, 16, 22 and 23). The vacuolar membrane, the tonoplast (Zirkle, 1937), is entire near the area of the nucleus (Photos 6, 7 and 8). The

nuclear boundary is often thick and in the region where it is in contact with the vacuole it is entire (Photos 1, 9, 22 and 23). Therefore, not only is the nucleus not enclosed by the vacuolar membrane but the vacuole and the nucleus are two unrelated cell organelles.

Most of the illustrations are drawn from non-budding cells for the reason that transfer of vacuolated cells to fresh media results in the early disappearance of the vacuolar membrane and then of the vacuole itself (Aswatha Narayana, 1956). Fresh media would stimulate the cells to divide and as a corollary changes should be expected in the nucleus also. Conklin (1925) remarks: " Some of these general differentiations of the cell are temporary and appear and then disappear with certain phases of cell activity. For example, chromosomes, asters and spindles differentiate during mitosis and dedifferentiate during intermitosis " (p. 544).

In the above context it may be that during mitosis the chromosomes may come to lie in the area of the cytoplasm originally occupied by the vacuole (Subramaniam, 1946). It is for the above reason that the tentative conclusions drawn in this paper relate specifically to non-budding cells in which well-formed boundaries separate the vacuole and the nucleus from each other as well as the cytoplasm.

Regarding the question whether there are formed structures inside the nucleus, observations under dark ground illumination can at best supplement only those made with the phase contrast microscope. Since granules lying outside the nucleus could be distinguished clearly (Photos 10 and 11) one has to presume that the bodies seen inside the nucleus (Photos 17, 18, 19 and 20) have a reality in view of the unmistakable evidence in this regard presented from cells (Photos 2, 3 A and 3 B) observed under the phase contrast microscope.

Summary

1. Observations under dark ground illumination supplement those made with the phase contrast microscope.

2. When the nucleus is visible in a non-budding cell, the vacuole and the nucleus are delimited from each other by well-formed membranes.

3. Considerable variation in its structure is exhibited by the nucleus in cells of a population.

Acknowledgment

I am very grateful to Dr. M. K. Subramaniam for his active interest and encouragement.

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EXPLANATION OF PLATES

PLATE XI

PHOTO; 1–5. *Living cells under phase contrast.*—Photo 1. The nucleus lies outside the vacuole. Photos 2 and 3 A. The nuclear membrane encloses a grain. Photos 3 B, 4 and 5. Orientation of the nucleus in bivacuolate cells. Two granules are present inside the nucleus in 3 B and one in 4 and 5.

PHOTOS 6–11. *Living cells under dark ground illumination.*—Photos 6, 7 and 8. Vacuolar membrane appears luminous. Nuclear area (*NA*) indicated. Photo 9. Nucleus and vacuolar membrane. Photos 10 and 11. Nuclear boundary luminous. A pair of grains occur outside the nucleus.

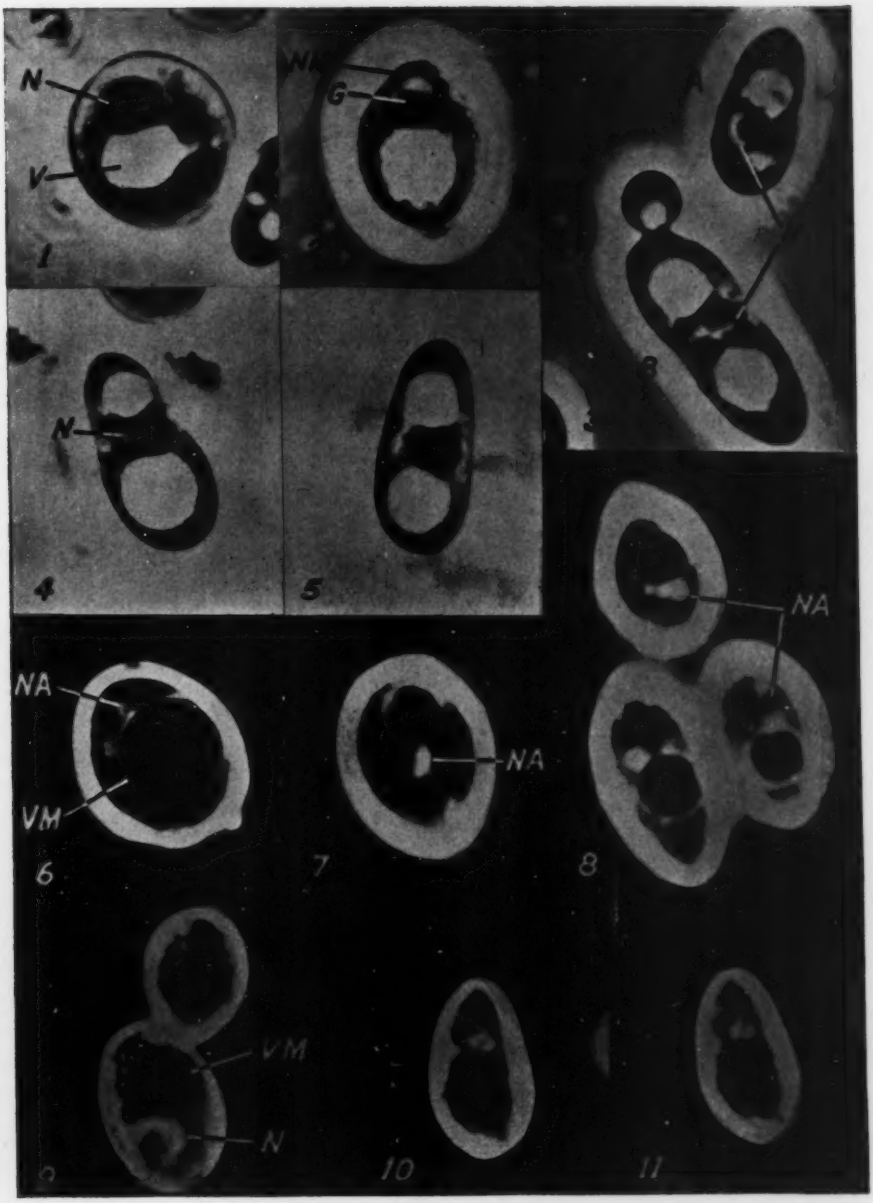
PLATE XII

PHOTOS 12–23. *Living cells under dark ground illumination.*—Photo 12. Nuclear membrane and the grain inside the nucleus appear luminous. Photos 13 and 14. Nuclear boundary indicated by luminous grains. Photos 15–21. Different types of orientation of the horse-shoe-shaped thickening of the nuclear boundary. The thickness of the two limbs are not uniform in 15, 17, 18, 19 and 20. Formed structures are present inside the nucleus in 17, 18, 19 and 20. Photos 22 and 23. The nuclear boundary (*NB*) is thick where it abuts on the vacuolar membrane.

KEY TO LETTERING

G, Grain inside the nucleus. *N*, Nucleus. *NA*, Nuclear area. *NB*, Nuclear boundary. *V*, Vacuole. *VM*, Vacuolar membrane.

Magnification, × *ca.* 2,500.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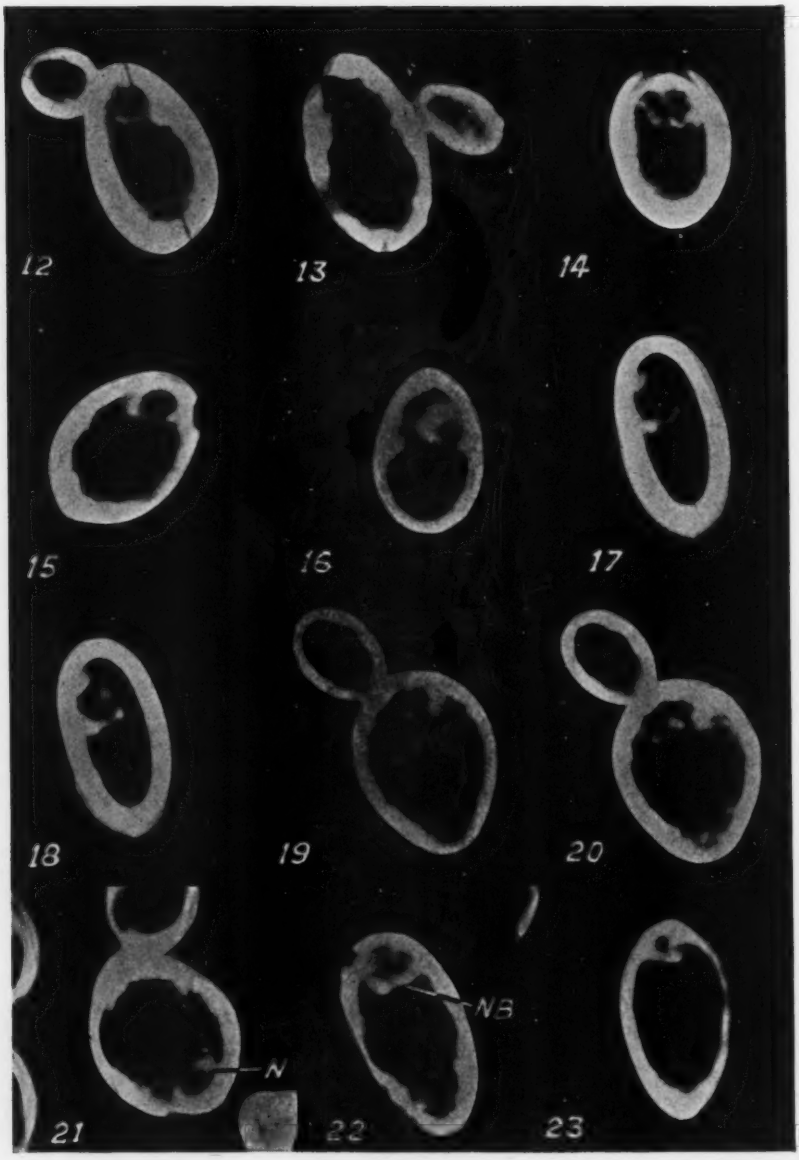
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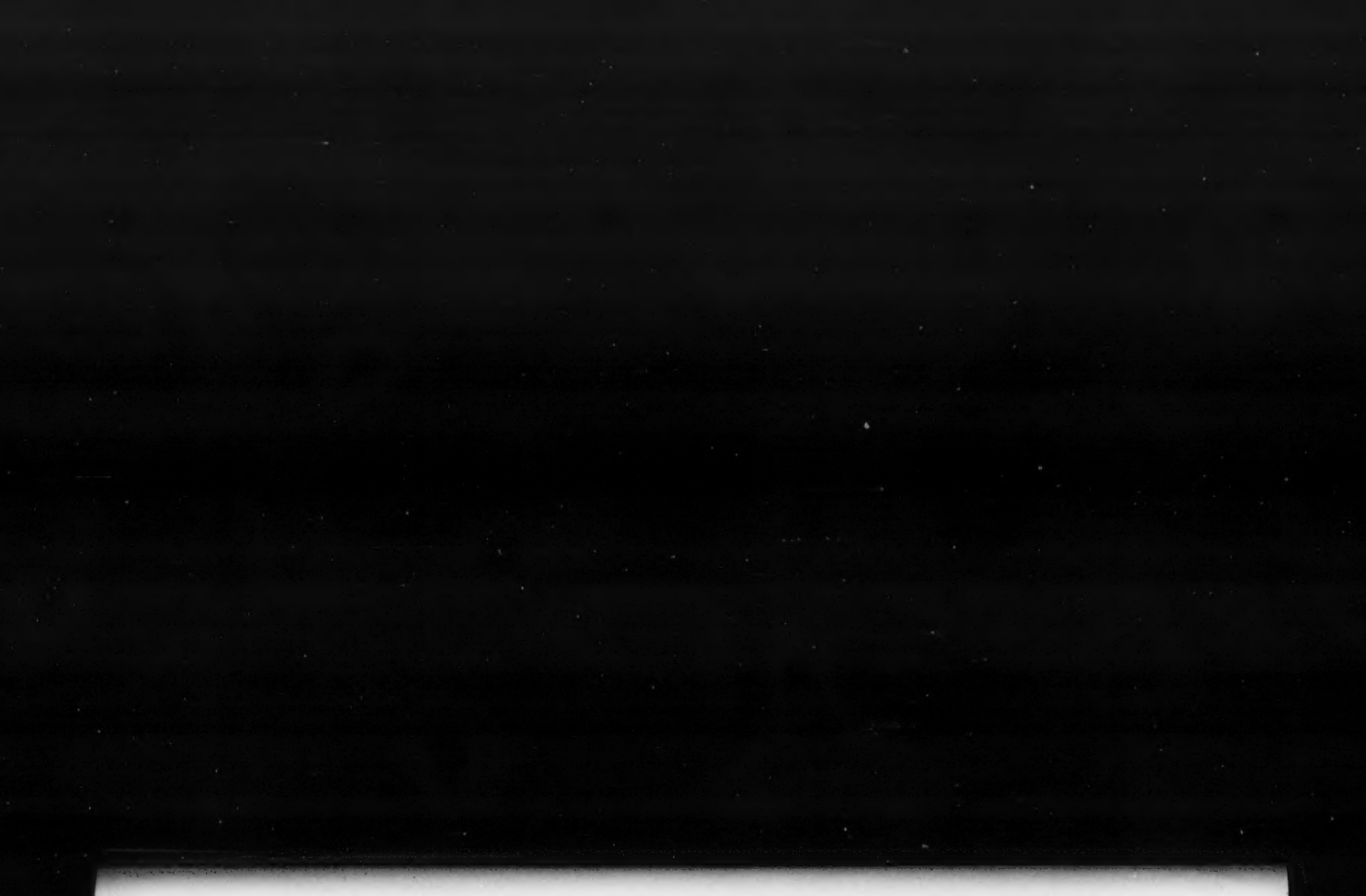

CONTRIBUTION TO OUR KNOWLEDGE OF THE PHYSIOLOGICAL ANATOMY OF SOME INDIAN HYDROPHYTES

IV. The Stem of *Sphaeranthus indicus* Linn.

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(Communicated by Dr. T. S. Sabnis, F.A.SC.)

Introduction

Sphæranthus indicus L., a member of the family Compositæ, is an annual, prostrate or sub-erect herb. It inhabits damp ground and the wet banks of ponds and lakes. The author has come across plants growing in actual water 1½ to 2 feet deep. These submerged plants, whose terminal portions protrude on the surface of water, however, flower only when the water recedes and they are exposed to air, although rooted in wet soil. The flowering season extends from December to January

The stem of this species is usually profusely viscous, glandular-hairy and bears prominent toothed wings. The leaves are obovate, profusely hairy and have a spinous-serrate margin. This description applies to the wetland form of the plant. Plants growing in deeper water, on the other hand, have stems that are glaucous, soft and spongy. The wings may be altogether absent or they may be represented by mere ridges on the surface of the stem. Portions of this submerged stem may be entirely leafless. Leaves, when borne, are narrow, almost glabrous and have an entire or distantly toothed margin. Terminal parts of submerged stems that come above the surface of water and the leaves borne on them are again softly pubescent. The deep water plants of *Sphæranthus indicus* L., thus, differ markedly from those growing on wetland (*cf.* Plate XIII, Photo 1: A and B). Indeed, it is not until one sees the protruding portions of the submerged stems and gradually follows the transition towards the margin of the lake, where water has recently receded and the plants, after exposure, have started flowering, that one is in a position to relate the two markedly different forms to the same species,

MATERIAL AND METHOD

The interesting morphological observations described above were observed by the author on the margins of the Munara Lake, a semi-natural reservoir of water, at Umred, at a distance of 30 miles from Nagpur, in the months of December and January when the plants are abundantly flowering. The material was fixed in formalin acetic alcohol. As in previous cases, the microtome method was found to be unsuitable for a detailed study of tissues. Figures in the text are camera-lucida sketches made by the author of hand-cut sections of the stem. Plate XIII (2 and 3) shows photographs of microtome sections intended to bring out the contrast between the general plan of structure of the two forms of stems studied.

DESCRIPTION

Anatomical investigation from the physiological point of view reveals a great contrast between the aerial portions of stems of the wetland plants and the submerged stems of plants growing in deeper water. This will be evident from the description given below. Lower portions of the former which are situated in wet soil naturally show an intermediate structure.

(A) *Aerial Portion of the Stem of the Wetland Form*

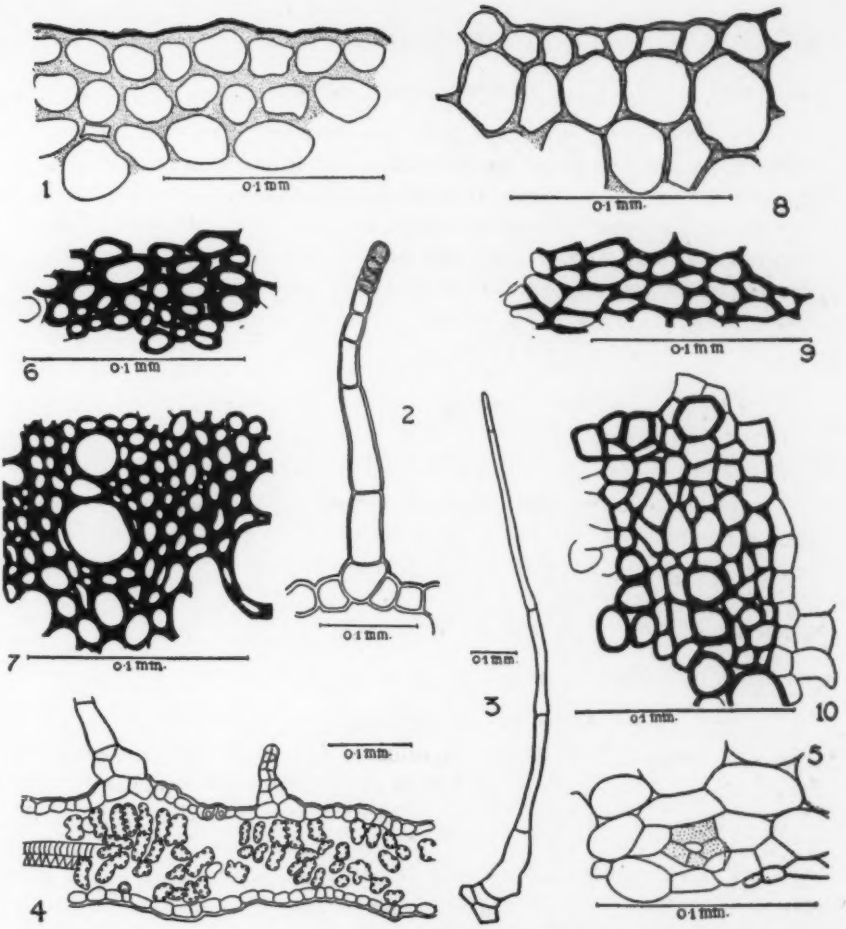
1. *Epidermis.*—Outer walls thickened; cuticle thin and papillose (Fig. 1). Stomata fairly abundant, situated in a level with the general epidermis; guard-cells much smaller than the ordinary epidermal cells. Hairs of two types. (i) *Glandular Hairs.*—Uniseriate, length varying, terminal three to four cells secretory (Fig. 2). (ii) *Covering Hairs.*—Very much longer than the glandular ones (Fig. 3); very abundant on the upper parts of the stem and on the peduncle of the inflorescence, rather rare on the lower parts.

2. *Cortex.*—Differentiated into three concentric zones.

(i) *Outer Cortex.*—Two to three layers of well-developed collenchyma (Fig. 1).

(ii) *Middle Cortex.*—Narrow, lacunar; two rows of schizogenous air passages which are oval in outline; cells roundish, thin-walled, chlorophyllose (Fig. 13).

(iii) *Inner Cortex.*—Two layers of thin-walled parenchyma; resin-ducts (Fig. 5) at regular intervals. Endodermis of large clear cells containing starch grains.



TEXT-FIGS. 1–10. *Sphæranthus indicus* L. Series of diagrams illustrating the contrast between the anatomical structure of the aerial part of the stem of the wetland form (A) and that of the submerged stem of the deep water form (B). All the figures, except Figs. 2 and 3, represent transverse sections. For explanation see the text.

3. *Pericycle.**—Heterogeneous; stereid bundles alternating radially with thin-walled parenchyma; sclerenchymatous fibres of the bundles well

* The author has been describing the region immediately encircling the vascular system under the commonly used term "pericycle". He is, however, aware of the fact that recent researches have thrown in doubt the existence of the pericycle in most angiospermic stems (*cf.* Esau, 1953). Eames and MacDaniels (1947) while accepting the claim that "no pericycle is present in the case of many angiospermic stems, because the fibres which were thought to make up of this layer belong to the phloem," add, "to what extent the 'pericyclic fibres' generally are phloem fibres is at present unknown. Study of the ontogeny of the region is necessary for the determination of the tissue to which the so-called 'pericyclic fibres' belong". Metcalfe and Chalk (1950) point out that "there is no general agreement concerning the precise meaning of the term 'pericycle'". The term "pericycle" in the present series of contributions may, therefore, be considered to carry a positional meaning rather than any histological significance.

developed, showing narrow cell cavities and pronounced lignification of the walls (Fig. 6).

4. *Vascular System.*—Vascular bundles of the sunflower type. Xylem fibres and vessels well developed (Fig. 7); phloem containing starch. Normal secondary growth due to vascular cambium.

5. *Pith.*—Thin-walled parenchymatous; lacunar; intercellular spaces fewer and smaller than those in the cortex (Fig. 12).

Wing.—Epidermis on both surfaces, cuticle thin; uni- and bi-seriate glandular hairs of varying length, covering hairs rare. Chlorenchymatous palisade-like cells forming the main body of the wing; intercellular spaces abundant (Fig. 4). A number of vascular bundles traverse the wing.

(B) *Submerged Stem*

1. *Epidermis.*—Cells thin-walled, cuticle absent; stomata fewer; hairs totally lacking.

Wing entirely reduced or represented by a stout emergence from the epidermis of the stem. The epidermis of this outgrowth is thin-walled and without cuticle. The core is formed of thin-walled cells not clearly differentiated into a palisade-like tissue.

2. *Cortex.*—Much more wider than in (A). Collenchyma of the outer cortex feebly developed (Fig. 8). Middle cortex forms the most conspicuous region of the stem (Fig. 11). It is profusely lacunar. The air-spaces are very large. The cells are thin-walled and radially elongated. They show the presence of chloroplasts. Inner cortex and endodermis as in (A).

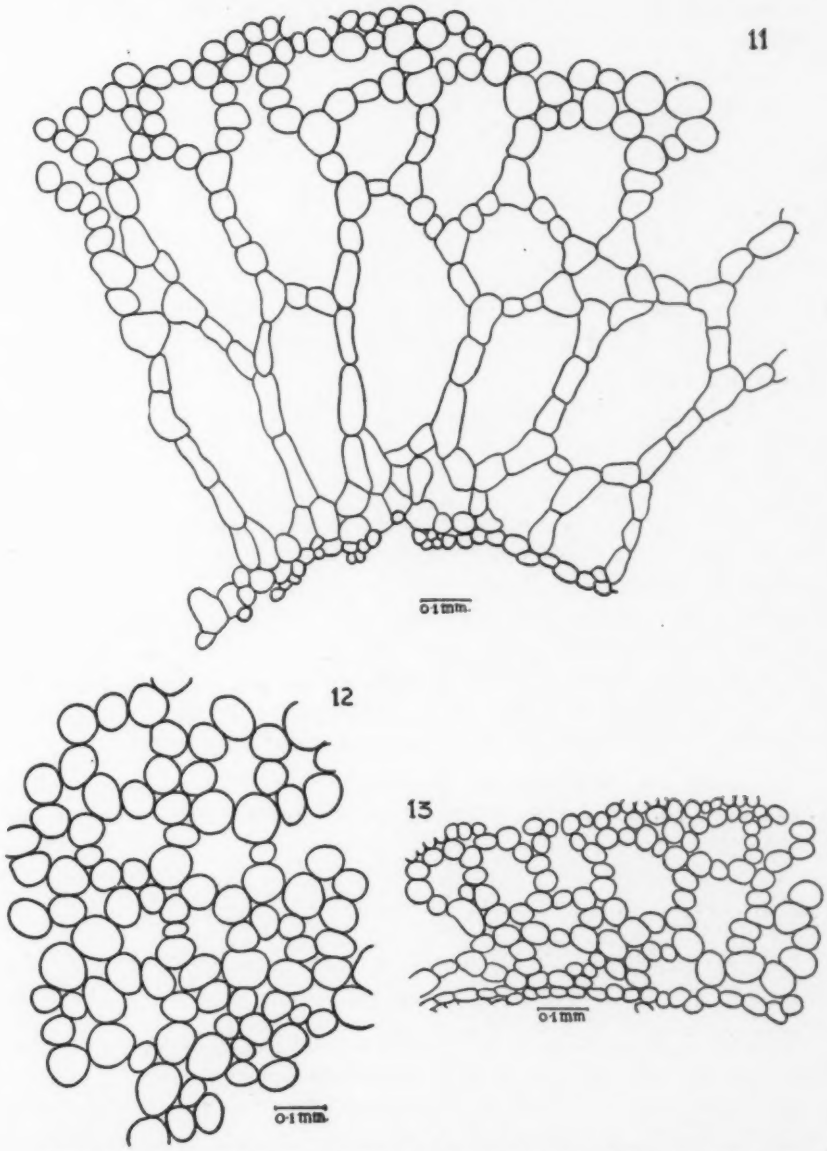
3. *Pericycle.*—Heterogeneous as in (A). The sclerenchymatous fibres of the stereid bundles have comparatively larger cell cavities and poorly lignified walls (Fig. 9).

4. *Vascular System.*—Conforms to that in (A). The walls of xylem fibres and vessels are thinner and less lignified (Fig. 10).

5. *Pith.*—Thin-walled parenchymatous, sparingly lacunar as in (A).

DISCUSSION

1. The foregoing account of the physiological anatomy of the stem of *Sphæranthus indicus* L. clearly brings out its amphibious character. A comparison of the structure of the aerial portion of the stem of the wetland form with that of the submerged stem of plants growing in deeper water reveals that plasticity of structure which is so characteristic a feature of the marsh plants. The form (B), as described above, differs from the form (A) both in



TEXT-FIGS. 11–13. *Sphæranthus indicus* L. Aerating tissues of the stem (T.S.). For explanation see the text.

its morphological as well as anatomical features. These characters of difference could be briefly enumerated as follows:—

(i) Absence of wings and hairs.

(ii) Absence of cuticle.

(iii) Feeble development of collenchyma.

(iv) Reduction in the thickening of pericyclic fibres.

(v) Reduction in the thickening of xylem fibres and vessel.

(vi) Elongation of cortical cells, enlargement of air-spaces and a consequent increase in the diameter of the cortex.

2. As compared with the other two composites, *viz.*, *Cæsulia axillaris* Roxb. and *Eclipta alba* Hassk., described previously by the author (Mirashi, 1954, 1955), *Sphæranthus indicus* L. shows a greater degree of adaptation to the watery environment. Aerating tissue in the form of a lacunar cortex is possessed by the stems of all the three species. *Eclipta alba* Hassk. further agrees with the present species in showing an increase in the diameter of the cortex on account of the elongation of its cells and enlargement of the air-spaces. But, in the development of the lacunar pith as an aerating tissue, in addition to the lacunar cortex, the feeble development of collenchyma and the poorer lignification of sclerenchymatous fibres and xylem vessels, *Sphæranthus indicus* L. has gone a step further towards adaptation to water. In addition, these anatomical features are further accompanied by the loss of some morphological features, as well, like the hairs and wings which are very characteristic of the aerial portion of the stem of this species.

3. Such an "epharmonic variation"—to quote the term used by Cockayne (1916) and adopted by Small (1919)—has been reported by Compton (1916) in the case of another composite, *Cnicus arvensis* Hoffm. This species owes its spiny condition to the excessive insolation and dryness of the original habitat of its ancestors. However, after submergence for a few weeks under water, it produced "a slender, leafless stem exactly like the woodless stem of a true aquatic". The development of such hydrophytic "epharmonic variations" in a family which has attained a high degree of specialization in adaptation to its terrestrial life is certainly interesting.

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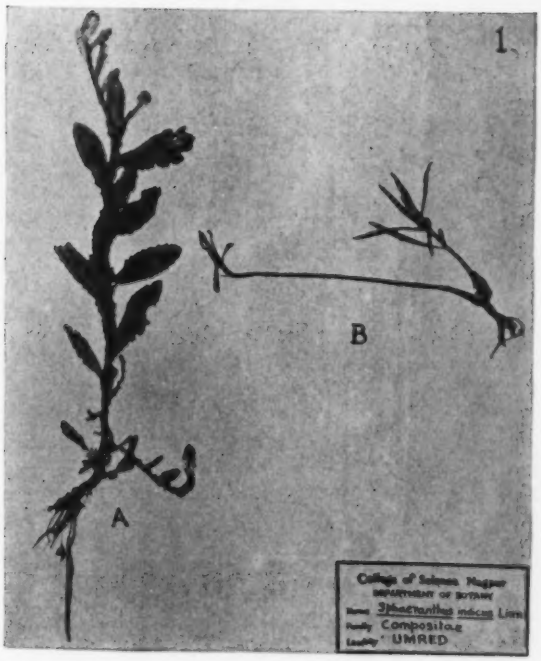
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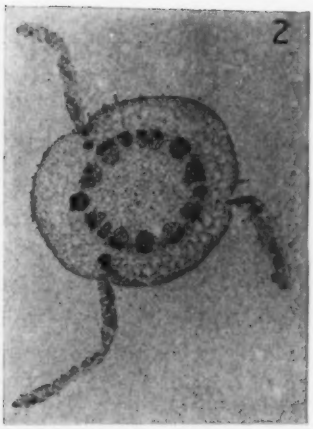
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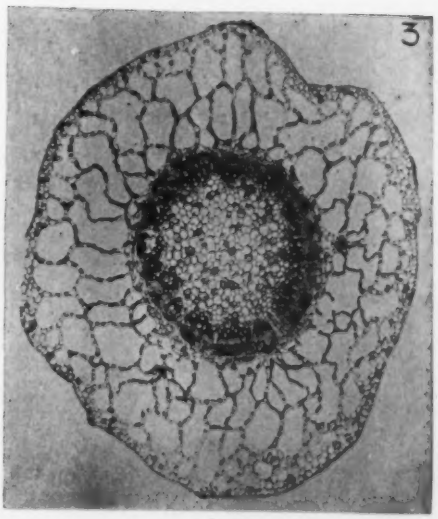
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Explanation of the Plate

Sphæranthus indicus Linn.

Photo 1. A—Wetland form ; B—Deep water form (about 2/5th natural size).

Photo 2. Transverse section of the aerial portion of the stem of the wetland form (A), ×16.

Photo 3. Transverse section of the submerged stem of the deep water form (B), ×16.

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ERRATUM

Vol. XLIV, No. 2 (August 1956)

Page 66, Line 21, for *G. papayæ* read *C. papayæ*.

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